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CANCER RESEARCH

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CANCER RESEARCH

A MONTHLY JOURNAL OF ARTICLES AND ABSTRACTS REPORTING CANCER RESEARCH

VOLUME 7 SEPTEMBER, 1947 NUMBER 9

Experimental Studies on the Pathogenesis and Histogenesis of Ovarian Tumors in Mice*

Min Hsin Li, Ph. D.** and W. U. Gardner, Ph. D.

(From the Department of Anatomy, Yale University School of Medicine, New Haven 11, Connecticut)

(Received for publication April 10, 1947)

The role of the anterior pituitary in the regulation of the gonadal function, and the reciprocal influence of the gonads on the pituitary gland have been established (81, 93). Gonadotrophic and ovarian hormones, under normal circumstances, maintain a state of antagonistic interaction (60). It is not difficult to understand, however, that certain conditions may arise that disturb the usual secretory activity of one or more of these endocrine glands thus resulting in hormonal imbalances. The prolonged exposure of animals of some species to estrogens results in the appearance of atypical growths or cancers in some genital tissues and in other organs (31). The nature of the neoplastic action of estrogens, whether estrogens act directly on the body tissues or indirectly through the disturbance of the production of pituitary hormones or otherwise, still awaits the results of further study.

The influence of pituitary hormones on the development of tumors has been studied by the use of animals given injections of different pituitary preparations, and by observing the effect of hypophysectomy on tumor-bearing animals or animals given carcinogenic substances. These investigations so far give little conclusive information (79). Recently, Pfeiffer and Hooker (69) obtained small local overgrowths of testicular interstitial cells in mice of the A strain that were given daily injections of pregnant mare's serum for several months. These growths resembled early stages in the development of the interstitial cell tumors that have been induced in estrogen-treated mice of this strain. The difficulty of obtaining purified and standardized gonadotrophic hormones, and the formation of antihormones have impeded adequate study of the long-term effects of gonadotrophins.

The present experiments are based on two principles: (a) the capability of the liver to inactivate ovarian hormones, *i.e.* estrogen and progesterone, when the hormones circulate through the hepatic portal system (6, 17, 35, 45, 46, 55, 77, 78, 91); and (b) the increase of intrinsic gonadotrophic hormones subsequent to castration as determined by bioassay of urinary, blood and hypophyseal gonadotrophins, and in experimental parabiosis of an intact with a castrated or roentgen-rayed animal (20, 21, 23, 28, 54). A condition of endocrine imbalance might be produced by the transplantation of ovaries into spleens of castrated mice. Such conditions should permit the study of continuous actions of endogenous pituitary gonadotrophins in ovarian tumorigenesis. Biskind and Biskind (4) reported that tumors developed in ovaries transplanted into spleens of 3 castrated female rats. Lipschütz and associates (52) observed only growth of lutein cells in some female guinea pigs with intrahepatic and intrasplenic grafts of ovaries.

Following the exposure of immature rats to roentgen irradiation (459 r or 574 r) directed to abdominal fields overlying the ovaries, Drips and Ford (18) observed hyperplasia and ingrowth of the germinal epithelium as well as the development of atypical, hyperplastic structures of epithelial cells that assumed an acinar arrangement and resembled "carcinomatous ovarian cystadenomata of the ovary of human beings." They found that such hyperplastic follicular structures occurred 4 months after irradiation, but more extensive growth had taken place in the rats that were killed 8 months later. The formation of granulosa-cell tumors, luteomas, and "tubular

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**Anna Fuller Fund Fellow in Anatomy. A part of this work was carried out under tenure of a fellowship given by the Chinese Government.

adenomas" in mice subsequent to similar applications of roentgen rays has been reported by Furth (29) and others (34, 85), and the histogenesis of the ovarian tumors has also been studied by several workers.

The present investigation is a study of the pathogenesis and the possible histogenesis of the ovarian tumors appearing in castrated mice bearing the intrasplenic ovarian transplants. A preliminary note on these tumors has been reported elsewhere (50).

MATERIALS AND METHODS

Inbred mice of the Strong A and C3H strains,[1] and hybrid mice (AC_3, AC_5)[2] were used. They were kept in an air-conditioned room and fed a commercial diet (Nurishmix) and water with occasional supplements of grain (a mixture of wheat, oats, and sunflower seeds) and calf-meal pellets. Six groups of experiments were set up (Table I): (a) 21 castrated male mice with homotransplants of an ovary into the spleen; (b) 52 castrated female mice with autotransplants of an ovary into the spleen; (c) 25 castrated female mice with subcutaneous autotransplants of an ovary into the right axillary region; (d) 12 intact male mice with subcutaneous homotransplants of an ovary into the right axillary region; (e) 9 castrated male mice with subcutaneous homotransplants of an ovary either into the right axillary region or into the abdominal region; and (f) 34 intact male mice with homo- or heterotransplants of an ovary into the right testis. Castration and grafting were done in one stage operations. Upon the removal of gonads the Fallopian tubes or sperm ducts and adipose tissues adjacent to the gonads were cauterized. The ovary was implanted into the spleen through a small incision made in the splenic capsule. The method of intratesticular transplantation of ovaries has been described (30). The ages of mice at the time of grafting varied from 1 to 3 months, except in the group (f) in which a few mice were operated upon at the age of 130 days. The donors of the ovaries for homotransplants or heterotransplants were usually younger than the recipients. The experimental animals received no treatment other than as mentioned.

Vaginal smears of the castrated female mice were taken at intervals during the experiment. Laparotomies were performed in some animals to check for adhesions and to determine growth of the graft. At autopsy the ovarian grafts, genital tissues, adrenal glands, kidneys, submaxillary glands, and pituitary glands were examined and preserved for histological examination. Mammary glands of a few mice were studied as whole mounts. Most of the ovarian grafts were sectioned serially and several areas from the ribbons were saved and stained with hematoxylin and triosin.

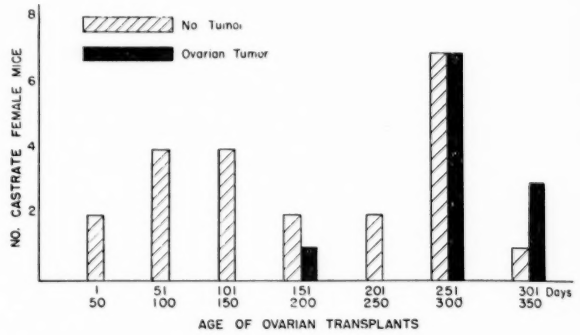


FIG. 1.—Age distribution of ovarian tumors in intrasplenic ovarian transplants in castrated male mice.

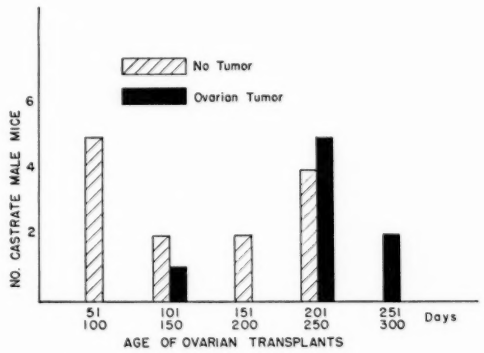


FIG. 2.—Age distribution of ovarian tumors in intrasplenic ovarian transplants in castrated female mice.

OBSERVATIONS

I. TUMORS IN INTRASPLENIC OVARIAN TRANSPLANTS

The ovarian transplants grew progressively in the spleens of castrated male and female mice and some were palpable through the abdominal wall at 3 months subsequent to grafting. At this time the transplants, observed at laparotomy, contained many large follicular cysts and they usually measured about 2 × 4 × 5 mm.

The earliest granulosa-cell tumor was discovered in a castrated male mouse bearing a 130 day old graft although most of the tumors occurred in grafts over 200 days old. Most ovarian tumors were larger than the non-tumorous grafts and protruded from the surface of the spleen; the largest measured 5 × 8 × 9 mm. in diameter. The tu-

[1]These mice were supplied by Drs. A. Gorbman, C. W. Hooker and L. C. Strong.

[2]AC_3 are first generation hybrid mice of the following parentage: C3H (low-tumor) ♀ × A ♂.

AC_5 are first generation hybrid mice of the following parentage: A (low-tumor) ♀ × C3H ♂.

mors were irregular in shape, reddish yellow, and some showed a few scattered hemorrhagic cysts as indicated by dark red spots (Fig. 4).

A. Castrated male mice bearing intrasplenic ovarian transplants.—Five granulosa-cell tumors, two pretumorous lesions of the same type and one mixed tumor— both granulosa and luteoma cells— were found among the 21 castrated male mice of the A strain (Table I). With one exception these tumorous grafts were more than 200 days of age.

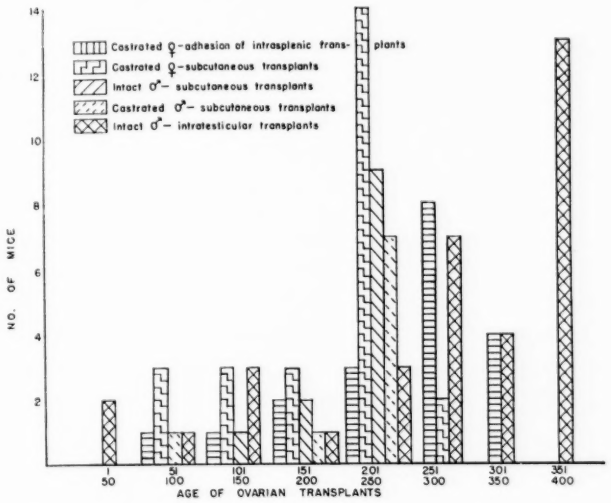


FIG. 3.—Age distribution of ovarian transplants in mice.

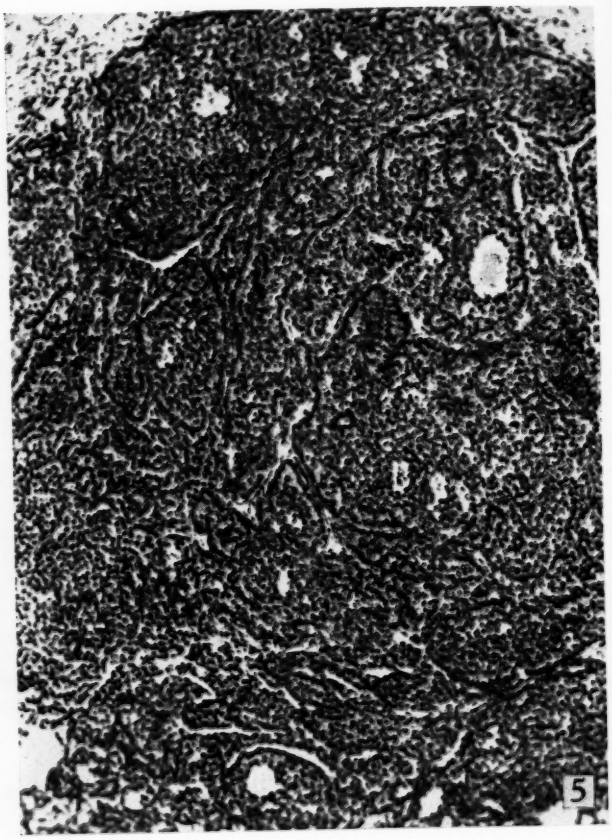
Among the 4 castrated male mice that survived over 200 days after the transplantation with no tumor growth only 1 showed viable ovarian tissue, a few primary follicles and some ovarian stroma constituting the small graft. The grafts in 2 other mice were composed of uterine tubal tissue or adipose tissue and in 1 animal no graft was found.

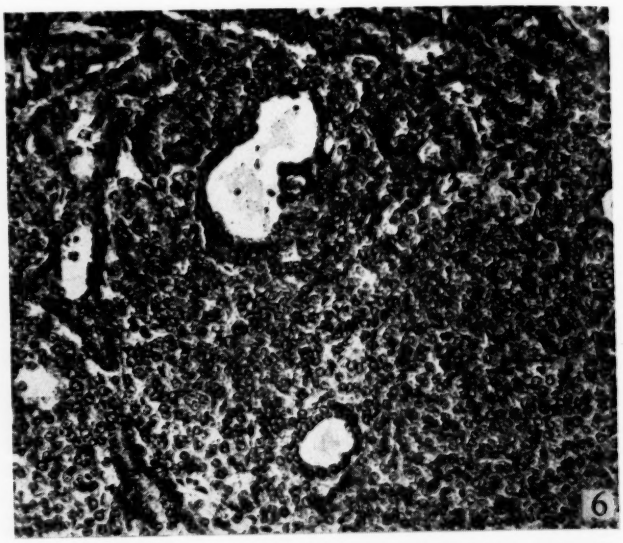
Nine mice that had no tumors of the transplanted ovaries were killed or died when the grafts were less than 200 days old. Two mice that had carried intrasplenic grafts for 170 and 198 days respectively had large transplants showing some ingrowth of the germinal epithelium and a disorganized stroma. The impression was given that, had the hosts survived for a longer period, the transplants would have become tumorous. The transplants in the two mice that had survived from 101 to 150 days were small, one could not be adequately examined because the animal had died sometime before autopsy and the other contained several large follicles and cysts of uterine tubal epithelium. Viable ovarian tissue was found in the 5 mice that had intrasplenic grafts for less than 100 days. The grafts differed in size but all contained follicles, either primary or both primary and secondary. The bulk of the grafts, however, consisted of enlarged interstitial cells with a pale staining cytoplasm. In only 1 of the later transplants were ingrowths of the germinal epithelium noted (Fig. 1).

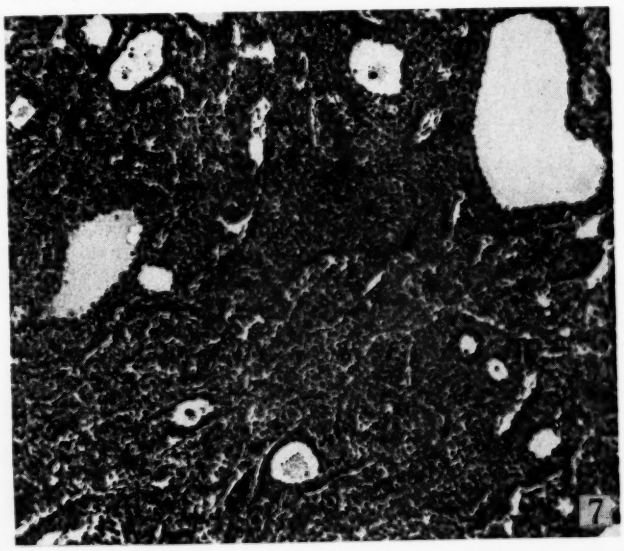
Microscopically, the granulosa-cell tumors consisted of cells with hyperchromatic vesicular nuclei. Most of the tumor cells were indistinguishable from the granulosa cells of normal graafian follicle; mitoses were frequent. In some areas the tumor cells invaded the splenic tissue although no metastases were observed. The tumor cells usually formed many somewhat dissociated masses and the arrangement of the cells in these masses varied considerably even in a single section of the same tumor. In some masses the tumor cells were diffuse and in others intermingled with thecal and interstitial cells; but most of the tumors contained extensive one-cell-layered cords that were more deeply stained than the adjacent tumor cells, and that were sometimes arranged in tubular structures (Fig. 5). Enlargement of the tubular spaces appeared to give rise to small and medium-sized cavities that usually contained a few degenerating cells and sometimes blood cells or coagulated fluid (Fig. 6). Also, folliculoid structure seemed to form by separation of a mass of tumor cells into smaller groups each surrounded by delicate connective tissue septa; the cavities resulted apparently by subsequent liquefaction of some of the centrally placed cells (Fig. 5, 7). A layer of spindle-shaped cells, suggesting thecal cells, was observed outside the ovarian follicle-like structures. Accumulation of the tumor cells and false ovocytes occasionally simulated the appearance of a cumulus oophorus (Fig. 7). Thus the granulosa-cell tumors were preponderently diffuse and folliculoid in pattern as featured by Barzilai (3) in human material. Different histological arrangements of the tumor cells co-existed in the same tumor. However, two tumors referred to as the 'pretumorous' lesions were composed of small groups of diffuse granulosa cells and a few hemorrhagic cavities.

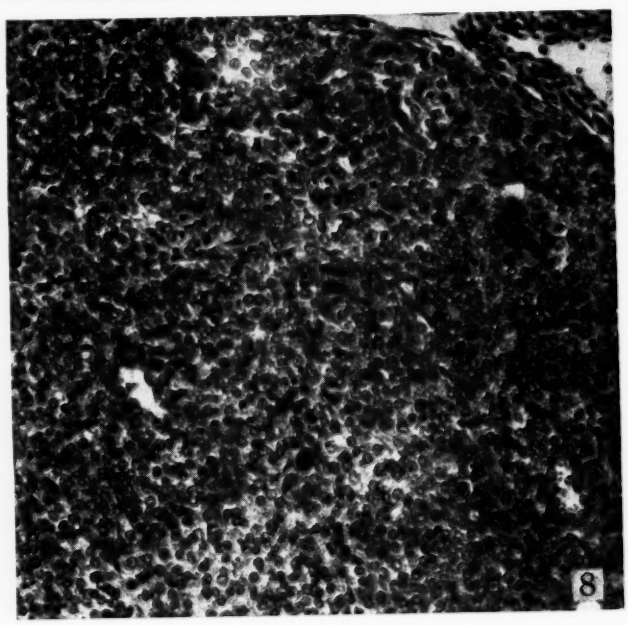
A large mass of lutein-like cells (luteoma) was observed in one of the granulosa-cell tumors, as well as many scattered lutein and interstitial cells. Some granulosa-tumor cells seemed to show transformation into lutein cells (Fig. 9). A description of luteomas will be given when the tumors in the castrated female group are described.

The proliferation and ingrowth of the germinal epithelium were observed in all these tumors and in some of the non-tumorous grafts. Local proliferation of the germinal epithelium with evidence of mitotic activity occurred frequently in the tumors, and cells with ellipsoidal nuclei simulating the cells of the germinal epithelium were often mixed with the adjacent tumor cells (Fig. 10).









FIGS. 4-8

The ingrowth of germinal epithelium formed groups of networks of tubules or cysts that sometimes contained blood cells as seen in the folliculoid structures. Many cells identified as granulosal, lutein, or interstitial cells, and primary ovocytes were observed among the network of epithelial ingrowths (Fig. 11). In some grafts the nuclei of the epithelial cells that lined the tubules appeared to become vesicular and the whole mass of epithelial ingrowth resembled the folliculoid formations (Fig. 12). An intimate connection between the epithelial ingrowths and cells of the tumors was discerned (Fig. 13).

The seminal vesicles and prostates were atrophied in the castrated male mice bearing intrasplenic ovarian grafts, and histological examination of the seminal vesicles revealed no indication of hormonal stimulation. The adrenal glands showed degeneration of the x-zone. No detectable hormonal effect on the kidneys was noted and the parietal layer of the Bowman's capsule was usually composed of squamous epithelial cells. The terminal tubules of the submaxillary glands were lined by low epithelial cells, but the percentage of total tubular and alveolar areas appeared to be equal.

B. Castrated female mice bearing intrasplenic ovarian transplants.—Four luteomas and 7 mixed tumors were found among the 33 castrated female mice of the A strain that showed no adhesion of the intrasplenic ovarian graft to the left uterine horn or to the adjacent peritoneum. Among the 19 ovariectomized mice of the same strain with vascularized adhesions of the ovarian transplant, 1 luteoma was observed in a mouse that had irregular estrous cycles during the latter part of the experimental period (Table I). Unmixed granulosa-cell tumors were not noted. The ages of the intrasplenic ovarian transplants are summarized in Fig. 2. The mean age of the grafts that became tumorous was greater in the females (278 days) than in the males (236 days).

Microscopically, the luteomas were composed of polyhedral, lutein-like cells with finely granulated, acidophilic cytoplasm, and vesicular nuclei; mitoses were rare (Figs. 14, 15). The luteoma cells formed small tubular or cord-like structures surrounded by delicate connective tissue septa and were usually assembled, as in the granulosa-cell tumors, into large masses that showed incomplete encapsulation. Blood capillaries and sinusoidal spaces were observed among the tumor cells, and tissue mast cells and pigment-containing cells occasionally occurred in some areas. Groups of large, probably lutein cells, cells having a large lightly staining cytoplasm, were found frequently in the tumors.

Seven tumors in this group of experiments were of mixed type consisting of elements of both granulosa cells and luteomatous tissue (Fig. 16). Most

TABLE I: TUMORS IN OVARIES TRANSPLANTED TO CASTRATED MICE

Site of transplant	Hosts	Number of mice	Ovarian tumors			
			Granulosa-cell	Luteoma	Mixed	None
Spleen	Castrated males	21	5 (224–268 days) 2? (130, 262 days)	.....	1 (225 days)	13 (75–268 days)
Spleen	Castrated females	33	.....	4 (153–284 days)	7 (266–346 days)	22 (25–334 days)
Spleen (adhesion)	Castrated females	19	.....	1 (262 days)	.....	18 (95–346 days)
Subcutaneous	Castrated females	25	1? (243 days)	.....	.....	24 (61–278 days)
Subcutaneous	Intact males	12	.....	.....	.....	12 (141–239 days)
Subcutaneous	Castrated males	9	.....	.....	.....	9 (64–233 days)
Testis	Intact males	34	.....	.....	.....	34 (32–370 days)

DESCRIPTION OF FIGURES 4 TO 8

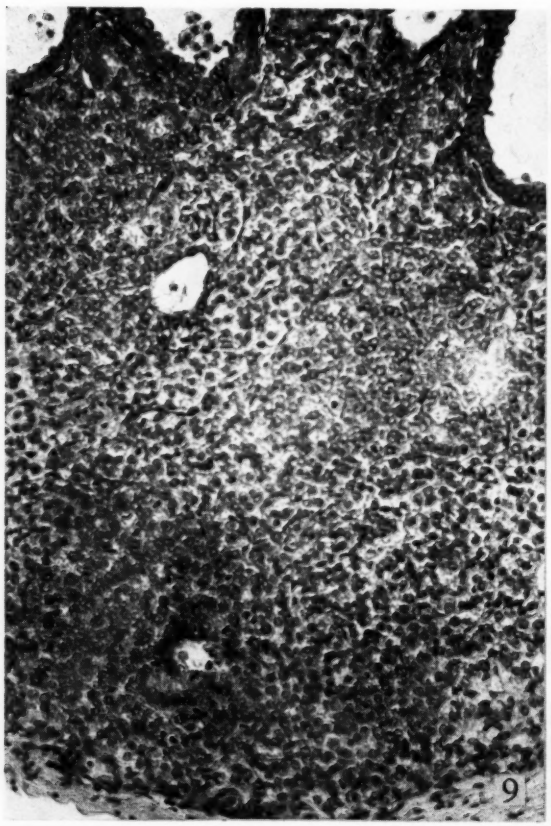
FIG. 4.—Granulosa cell tumor in an intrasplenic ovarian transplant in a castrated male mouse bearing the transplant for 268 days.

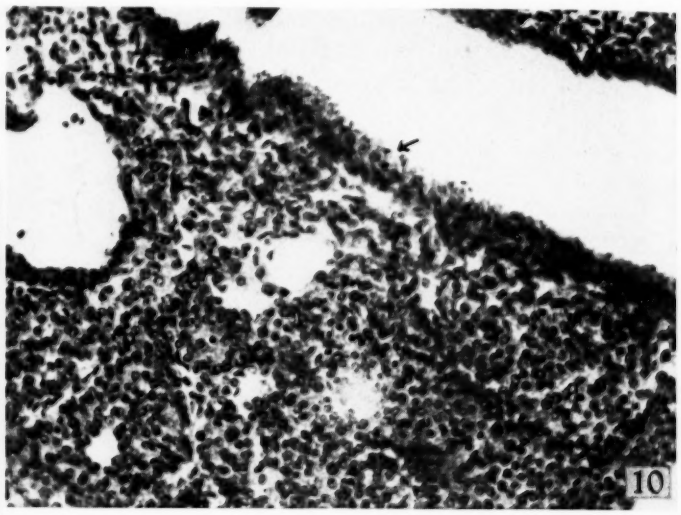
FIG. 5.—Section of a granulosa cell tumor as shown in Fig. 4. Mag. × 105.

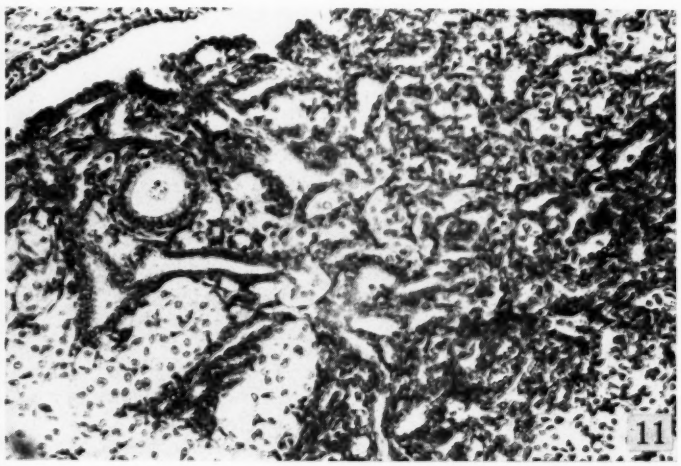
FIG. 6.—Granulosa cell tumor in an intrasplenic ovarian transplant (247 days) in a castrated male mouse, showing folliculoid structures. Mag. × 192.

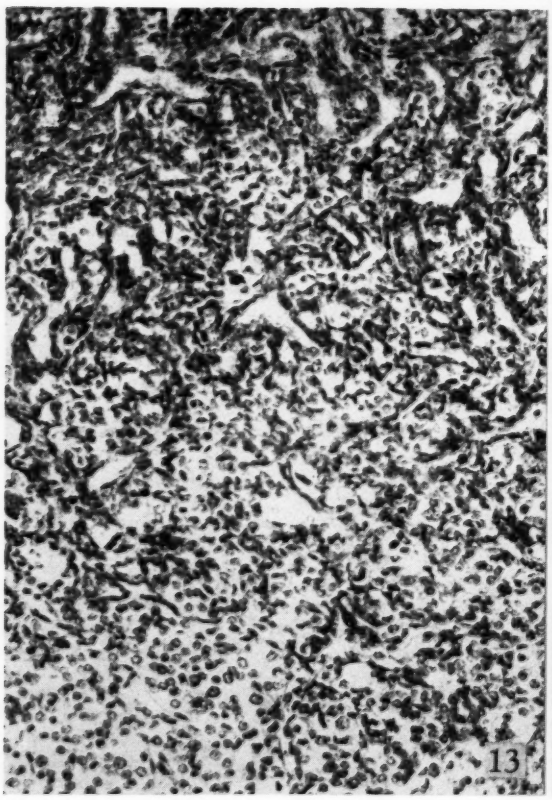
FIG. 7.—Folliculoid structures in a granulosa cell tumor resembling ovarian follicles. This tumor developed in an intrasplenic ovarian transplant (225 days) in a castrated male mouse. Mag. × 99.

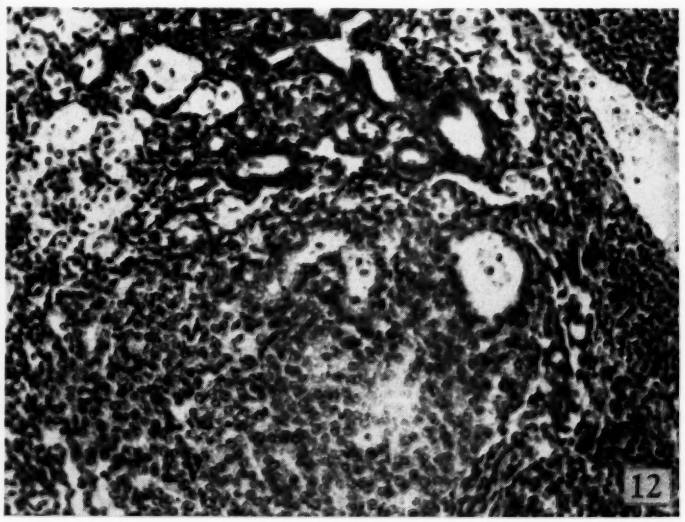
FIG. 8.—A diffuse mass of granulosa tumor cells showing mitotic figures. This tumor developed in an intrasplenic ovarian transplant (273 days) in a castrated female mouse. Mag. × 192.











FIGS. 9-13

granulosa-cell tumor masses exhibited the folliculoid pattern as described above for the castrated male mice. Transformation of granulosa cells of the tumors into luteomas appeared to take place in some of the masses of the former type (Fig. 17). Many granulosa cells showed vague cell boundaries indicating that they were becoming luteinized.

Proliferation and ingrowth of the germinal epithelium were observed in all of these tumors. The luteoma cells were sometimes in direct contact with the tubular ingrowth of the germinal epithelium and sometimes intermingled at the borders of masses of less differentiated granulosa tumor cells (Fig. 18).

Four castrated female mice that had tumors of the intrasplenic ovarian transplants showed estrous vaginal smears; 2 early and 2 in the latter part of the experiment. No estrous smears were obtained from the other 8 ovariectomized mice that had tumors in the intrasplenic ovarian transplants; their uteri were small, averaging about 37 mgm. Alveolar formation was not observed in the mammary glands of two animals that had viable intrasplenic ovarian transplants. The nuclei of the stromal cells of the endometria from the castrated females with the intrasplenic ovarian tumors were generally small, dense, and spindle-shaped, resembling those found in the uteri of castrated mice (44). The x-zone of the adrenal glands was absent and areas with compact groups of rounded or fusiform cells were found in the immediate subcapsular region of the cortex of almost all adrenals from mice of this group. The latter condition was noted only in the castrated female mice. Similar changes associated with the formation of adrenal cortical carcinoma have been described by other workers in spayed female mice of the extreme dilution strain (90). As in the castrated males, the parietal layer of the renal capsules was usually composed of low epithelial cells. The relative extent of tubular and alveolar areas in the submaxillary glands were equal, but the terminal tubules, in most cases, essentially consisted of columnar cells. The animals with adhesion of the intrasplenic ovarian transplants had submaxillary glands of the female type.

Fourteen of the 22 intrasplenic ovarian transplants that did not become tumorous were less than 250 days old, and only one tumor was noted in a younger graft (153 days). Irregular estrous smears were obtained in 12 of the 22 castrated female mice, and the average uterine weight of these 12 female hosts was 50 mgm.

The stroma of all but one of the nontumorous ovarian grafts consisted predominantly of large lutein-like interstitial cells arranged in masses, in islands separated by sinusoids, or in cords separated by sinusoids, or ingrowing strands from the germinal epithelium. Usually these cells had a granular eosinophilic cytoplasm but sometimes the cytoplasm showed little affinity for the stains. Almost all of the grafts contained a few small or primary follicles. Large follicles were found in all but one of the grafts less than 250 days old, and were rarely noted subsequently. Hemorrhagic follicles or cysts were present in all but 2 of the grafts. The first evidence of ingrowth of the germinal epithelium was noted in an ovary that had been grafted in the spleen for 110 days. None of the other 11 grafts less than 200 days old showed epithelial ingrowths whereas 5 of the 10 grafts over 200 days of age showed ingrowths of the germinal epithelium. These epithelial ingrowths were adjacent to areas of lutein-like stromal cells, and in some places the continuity was such as to suggest the origin of the latter by differentiation from the former.

No typical corpora lutea were noted but 5 of the 12 intrasplenic ovarian grafts less than 200 days old contained structures considered to be luteinized follicles, some of which closely resembled old corpora lutea. Lutein-like cells were noted along the walls of some large follicles indicating that the granulosa cells were undergoing transformation. There was no evidence of cyclic formation of

DESCRIPTION OF FIGURES 9 TO 13

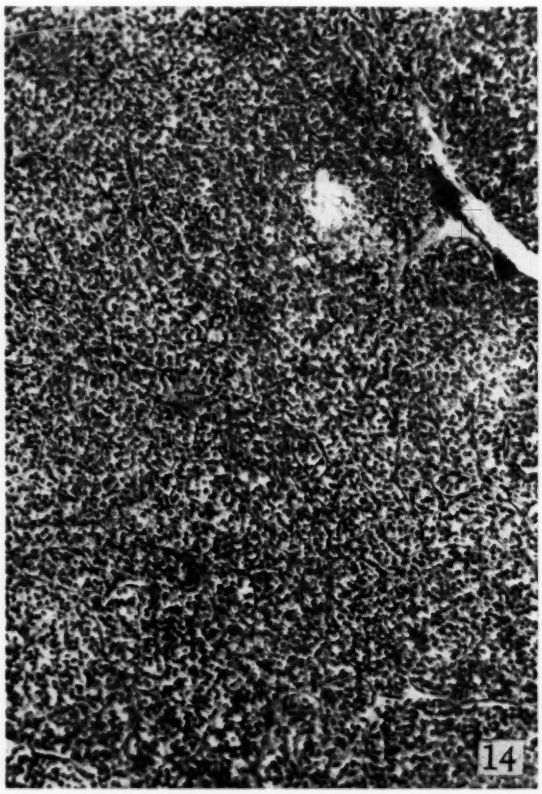
FIG. 9.—Partial luteinization of granulosa tumor cells. This tumor developed in an intrasplenic ovarian transplant (247 days) in a castrated male mouse. Mag. × 168.

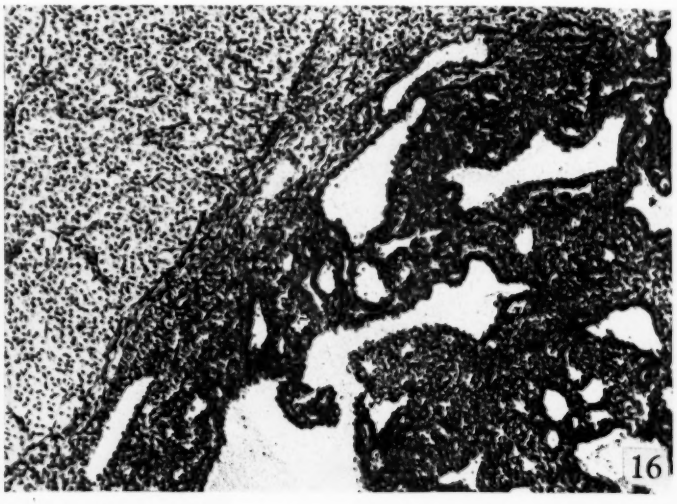
FIG. 10.—Local proliferation of germinal epithelium in an intrasplenic ovarian transplant (225 days) in a castrated male mouse. Note the mitotic activity (arrow indicated) and the close association of the germinal epithelium with granulosa tumor cells. A bursal space separated this part of transplant from the splenic tissue (above). Mag. × 178.

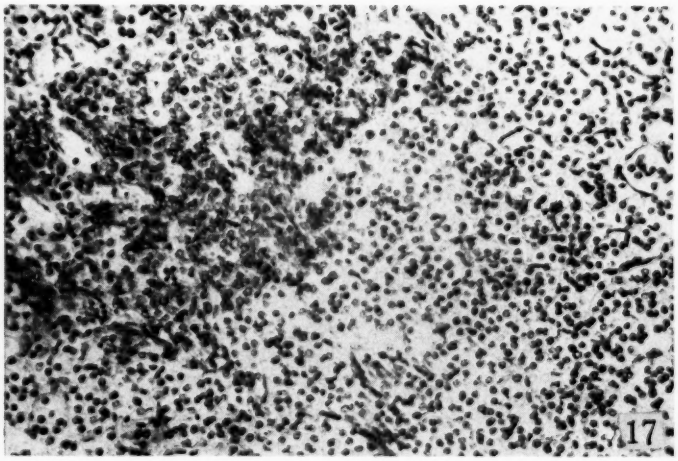
FIG. 11.—Tubular ingrowth from germinal epithelium in an intrasplenic ovarian transplant (225 days) in a castrated male mouse. Note the intimate relationship of the primary ovocyte, lutein cells and follicle cells with the network of epithelial ingrowths. Mag. × 171.

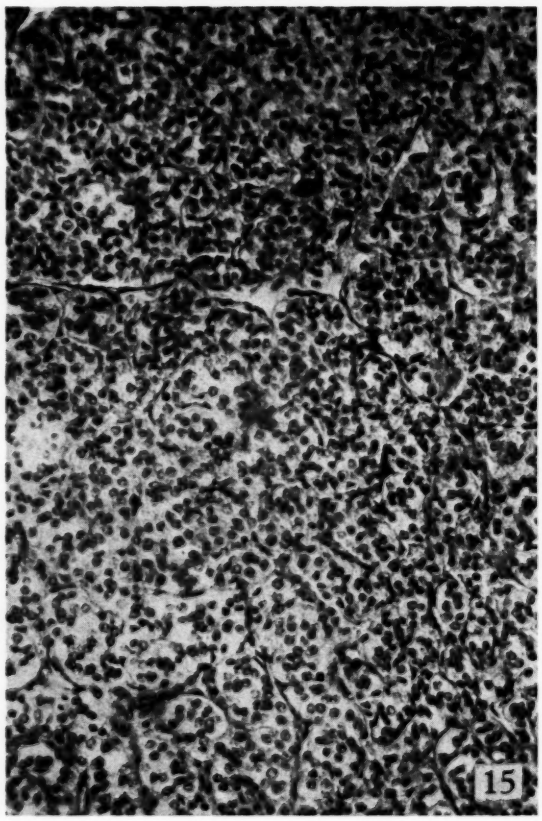
FIG. 12.—Section showing the close associations of tubular ingrowths with granulosa tumor cells, and the transplant with the splenic tissue at right. Same tumor as illustrated in Fig. 10. Mag. × 178.

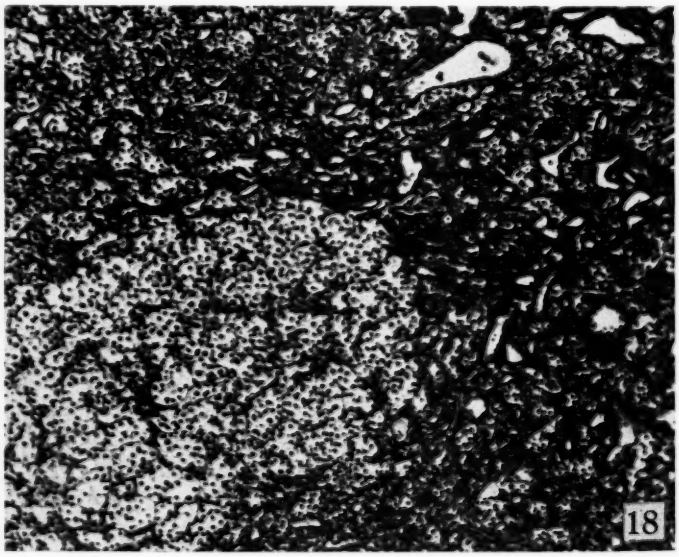
FIG. 13.—Section showing indication of transformation of granulosa tumor cells from tubular ingrowths of the germinal epithelium. This tumor developed in an intrasplenic ovarian transplant (262 days) in a castrated male mouse. Mag. × 192.











FIGS. 14-18

corpora lutea such as has been found in ovaries of intact mice. The corpora lutea-like structures were not observed in the intrasplenic grafts in the males. In this respect, in the somewhat delayed appearance of epithelial ingrowth into the stroma and of tumor formation, and in type of tumor formed, the grafts of the males and females differed appreciably.

The intrasplenic ovarian transplants in castrated female mice that had adhesion of the spleen, or graft to the adjacent body wall, uterus or kidney capsule, with one exception, were quite different from those in the mice that had no adhesion (Fig. 3). Almost all of the grafts contained a greater number of large and small ovarian follicles and corpora lutea, or follicles with completely luteinized cells (Table II). Although lutein-like stroma cells were apparent in several of the grafts they made up but a small part of the stroma in most instances, a more normal fibrous stroma predominating. Only in one mouse (a 262 day old transplant) was a marked overgrowth of lutein-like cells noted; they were sufficiently anaplastic in appearance to be classified as luteomatous. One of the grafts had largely regressed (270 days) and one consisted only of stroma and large sinusoids (324 days). Ingrowth of the germinal epithelium occurred in only 2 grafts, one of which was the luteoma.

TABLE II: INTRASPLENIC OVARIAN TRANSPLANTS IN CASTRATED FEMALE MICE WITH ADHESIONS TO STRUCTURES DRAINED BY CAVAL SYSTEM OF VEINS*

Age group (days)	No. of mice	Number of ovarian transplants with: Large follicles	Small follicles	Corpora lutea	Hemorrhagic cysts	Luteinized stroma	Germinal epithelium ingrowth
51–100	1	1	1	0	0	1	0
101–150	1	1	1	1	0	1	0
151–200	2	2	2	1	1	2	0
201–250	3	3	3	3	0	2	0
251–300	8	6	6	1	3	5	2
301–350	4	3	3	2	1	1	0

*Studies were made on test sections removed from several different areas of the transplants.

In summary, the presence of adhesions was associated with the more normal constitution of the ovarian tissues, namely, the occurrence of follicles and corpora lutea and a low incidence of ingrowth of the germinal epithelium.

II. SUBCUTANEOUS TRANSPLANTS OF OVARIES

A. Castrated female mice bearing subcutaneous ovarian transplants.—Among the 25 castrated female mice of the A strain with ovaries transplanted subcutaneously, one small granulosa cell-like growth was observed upon histological study (Fig. 19). The mouse was the only one that was operated upon during pregnancy and carried the graft for 243 days. Masses of granulosa cells surrounded many irregular hemorrhagic cavities and mitotic figures were frequent. Lutein cells, luteinized granulosa cells and one primary follicle were also found in this transplant. The ingrowths of the germinal epithelium formed many tubular or cystic structures that merged into the tumor-like masses. No estrous cycles were noted in this mouse during the first month after the operation, but cornified vaginal smears were obtained in the latter part of the experiment. The uterus weighed 153 mgm. at autopsy.

The other 24 castrated females carried the subcutaneous ovarian transplants for periods of 61 to 278 days. Although most of them were killed 8 months after the operation, the grafts usually were not as large or as well developed as those in the spleen, and in 7 of the 24 hosts ovarian tissues could not be definitely identified. The subcutaneous, fibro-fatty tissues encroached upon the grafts except where a capsule lined by the germinal epithelium intervened. Small masses of lymphoid tissue or areas of diffuse lymphoid tissue occupied parts of the ovarian grafts and of the adjacent tissues. Most of the ovarian grafts contained primary and maturing follicles, and luteinized follicles, some with hemorrhagic centers (Fig. 20). The stroma was composed of lutein-like interstitial cells, and in 4 grafts large areas, apparently of interstitial cells or luteinized follicles, were necrotic. Degeneration of this type has been noted in the ovaries of old mice but was not observed in the intrasplenic ovarian transplants. Ingrowth of the germinal epithelium was noted in

DESCRIPTION OF FIGURES 14 TO 18

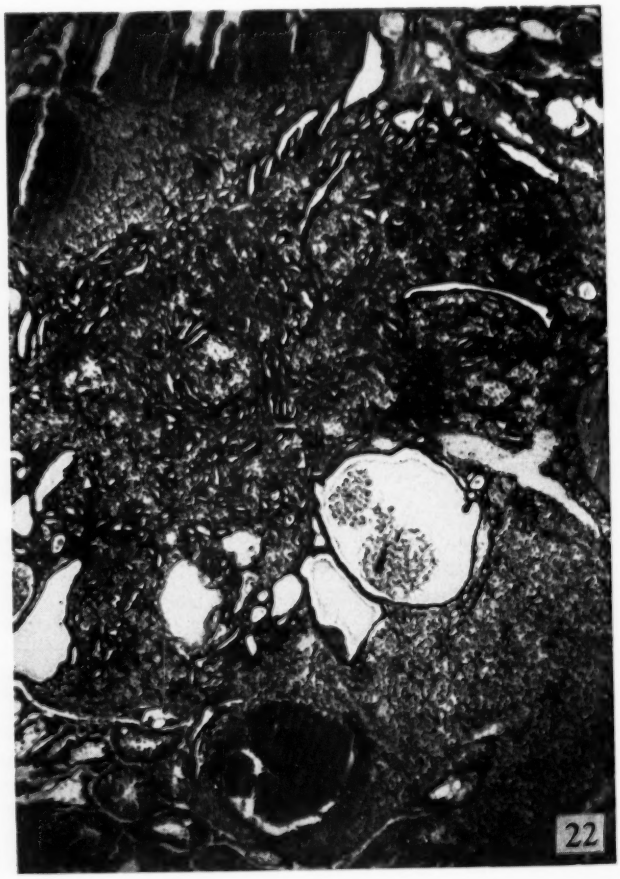
FIG. 14.—Luteoma in an intrasplenic ovarian transplant (284 days) in a castrated female mouse. Mag. × 93.

FIG. 15.—Higher magnification of Fig. 14. Mag. × 186.

FIG. 16.—A mixed tumor consisting of granulosa cells (right) and luteoma cells (left). This tumor developed in an intrasplenic ovarian transplant (334 days) in a castrated female mouse. Mag. × 89.

FIG. 17.—Section showing indication of transformation of granulosa tumor cells into luteomatous tissue in an intrasplenic ovarian transplant (273 days) in a castrated female mouse. Mag. × 178.

FIG. 18.—Section showing intimate relationship between tubular ingrowths of the germinal epithelium and luteoma cells. Same tumor as illustrated in Fig. 17. Mag. × 93.



FIGS. 19-22

3 of the grafts in addition to the 1 that had become tumorous.

Estrous vaginal smears were obtained from these animals and the average weight of their uteri was 60 mgm. Some development of alveoli was maintained in the mammary glands. The adrenal glands had no x-zones and in the subcapsular region of the adrenal cortex there were compact groups of deeply stained cells similar to those observed in the group of castrated female mice with the intrasplenic ovarian transplants. Some cuboidal cells were found in the parietal layer of the Bowman's capsules. The submaxillary glands exhibited the female type of histological appearance with more alveolar than tubular structures and the terminal tubules were mainly composed of cuboidal cells.

B. Castrated male mice bearing subcutaneous ovarian transplants.—Nine castrated male mice of the A strain had ovaries placed in the subcutaneous tissue and 8 were killed 173 to 233 days later (Fig. 3). Ovarian tissue was not found in the sections prepared from 3 of the grafts; they consisted of cysts of the ovarian bursae or oviducts. Some ovarian tissue may have been present at levels other than those at which the sections were removed, although several sections were taken from different parts of each transplant.

The one transplant, removed 63 days after grafting, contained many large follicles, some cystic but still lined by granulosa cells, and several smaller follicles. Corpora lutea were not present. The ovary was largely surrounded by a bursa-like cavity. The germinal epithelium had not invaded the ovary. The 173 to 233 day old transplants from 5 mice contained a few large follicles or follicular cysts, some of which contained blood and a few small follicles. Some small follicles contained partially luteinized granulosa cells. The stroma consisted of large cells with a pale-staining cytoplasm and was broken up by extensive ingrowth of cells from the germinal epithelium (Fig. 21). In at least 2 of the ovarian transplants small areas of stroma composed of lutein-like cells were reminiscent of the intrasplenic transplants in the castrated mice, although in almost all instances the grafts in the subcutaneous tissue were much smaller.

Atrophy of the seminal vesicles and prostates were observed in these castrated male mice. The mammary glands showed estrogenic stimulation with some growth of alveoli; and the submaxillary glands exhibited female characteristics. Groups of deeply stained small cells occurred in the subcapsular region of the adrenal cortex and no x-zone was present. In some Bowman's capsules the parietal lamina consisted partially of cuboidal epithelial cells.

C. Intact male mice bearing subcutaneous ovarian transplants.—The ovarian transplants of 8 of the 12 intact male mice bearing subcutaneous ovarian grafts for 140 to 239 days were examined histologically (Fig. 3). All of the grafts were small and were partially surrounded by bursa-like cavities. They contained few to many small and rarely a medium sized follicle. The stroma was composed primarily of compact fibroblast-like cells and "wheel cells" with small nuclei containing several aggregates of chromatin; stroma such as is found in the ovaries of hypophysectomized mice. A few "brown" cells and rarely a large cell with a clear cytoplasm were also observed in the stroma. Although the germinal epithelium was thick in many places, definite tubular ingrowths into the transplants were not noted. The small size of the grafted ovaries, absence of large follicles or corpora lutea, and the appearance of the stromal cells all indicated that the ovaries were but slightly stimulated by the gonadotrophic hormones of the hosts. In general, there were no appreciable effects of ovarian hormones in the male genital organs, adrenals, kidneys, and submaxillary glands.

III. Intratesticular Transplants of Ovaries

Thirty-four mice, 13 of the A strain and 21 hybrids (AC_3, AC_5), carried intratesticular ovarian grafts for 32 to 355 days. Twenty-four of the mice were killed when the grafts were over 250 days old, and 13 grafts were over 350 days old (Fig. 3).

The two grafts removed when less than 50 days old were small, were surrounded by bursa-like spaces, and contained chiefly follicles at several

DESCRIPTION OF FIGURES 19 TO 22

Fig. 19.—Granulosa cell-like growth, with evidence of epithelial ingrowth, in a subcutaneous ovarian transplant (243 days) in a castrated female mouse. Mag. × 42.

Fig. 20.—Subcutaneous ovarian transplant (242 days) in a castrated female mouse showing ovocyte, tubular ingrowth, hemorrhagic cyst, interstitial cell stroma, and necrotic areas. Mag. × 100.

Fig. 21.—Subcutaneous ovarian transplant (233 days) in a castrated male mouse showing follicle, tubular ingrowth, and interstitial cell stroma. Mag. × 100.

Fig. 22.—Intratesticular ovarian transplant (351 days) in an intact male mouse showing follicular cysts, hemorrhagic cysts, tubular ingrowth, and interstitial cell stroma. Mag. × 42.

stages of development. The older grafts were similar to one another in most respects and will not be described separately. All of the grafts, except one that was composed entirely of interstitial cells (315 days old), were covered in part by a bursa-like space or spaces lined by columnar or cuboidal epithelium, apparently the persisting germinal epithelium. Epithelial extension from the surrounding bursa occurred in most of the grafts over 250 days old and tended to increase with advancing age (Fig. 22). The epithelial extensions grew only into the ovarian tissue and not out into the testis although both sides of the bursa were lined by cells that appeared to be identical. The germinal epithelial cells did not grow into the testicular stroma. The epithelial ingrowths resembled those observed in grafts at other sites in castrated male and female hosts. In several grafts the tubular ingrowths of germinal epithelium had become cystic and sometimes contained blood. The cells lining the cysts sometimes contained hemosiderin, and in places resembled interstitial cells and macrophages.

Most of the grafts contained several follicles of different sizes. A few follicles contained blood and the cells lining the walls were somewhat luteal. No corpora lutea or completely luteinized follicles were found.

The greater part of most of the grafts consisted of a stroma composed mainly of large, lightly stained cells with definite cell membranes, and of large cells containing a brownish pigment scattered among small blood vessels and sinusoids, and fibroblastic elements. Such tissue has been classed as interstitial in the ovarian grafts at other sites.

A number of the ovarian transplants, especially the older ones, contained small areas of cord-like or solid follicle-like structures of granulosa cells somewhat reminiscent of the granulosa-cell tumors. The scarcity of mitotic figures, the small size of the areas, and well organized stroma led to the impression that they should not be designated as tumorous. The impression was gained, however, that ovarian grafts in the testes might occasionally become tumorous.

The mammary glands in the male hosts with ovarian transplants showed a partially developed duct system, although kidneys, submaxillary glands, and other genital organs showed normal male characteristics. No x-zone was present in the adrenals and the growth of compact small cells in the subcapsular region of the adrenal cortex with occasional mitotic figures was found in the hybrid male mice. In mice of the A strain, however, this condition was observed in the castrated females and in castrated males bearing subcutaneous ovarian grafts.

DISCUSSION

I. Pathogenesis of the Ovarian Tumors

The high incidence of granulosa-cell tumors in women at or just preceding the menopause has been noted by several workers (15, 71, 80, 84, 87, 88), and the amenorrhea that was often ascribed to "the menopause" is now considered by Dockerty (15) as the first symptom of a granulosa-cell tumor. In presenting a case of granulosa-cell tumor in a 51 year old woman, Voigt (89) inferred that an overproduction of the gonad-stimulating hormone after the ovaries lost their inhibiting effect upon the anterior pituitary, might play a part in the formation of the tumor. Recently, Biskind and Biskind (4) reported three granulosa-cell tumors in rats' ovaries transplanted into the spleen and they suggested that the development of the tumor is due to the inactivation of ovarian estrogen by the liver, allowing the protracted stimulation of the pituitary to act on the ovary. These intrasplenic ovarian growths, however, have been questioned as "true blastomas" (80).

The present experiments provide evidence that an endocrine imbalance between the pituitary gonadotrophins and ovarian hormones is responsible for the development of granulosa-cell tumors and luteomas in mice. The increase of endogenous gonadotrophic hormones in castrated mice is manifested by the rapid growth of the ovaries and by the formation of hemorrhagic follicles shortly following the intrasplenic transplantations. The formation of hemorrhagic follicles (blood points), as Zondek (92) has pointed out, is a most conspicuous and impressive reaction in the immature mouse's ovary to the administration of anterior pituitary hormones. Furthermore, the increase of intrinsic gonadotrophins subsequent to castration has been shown by bioassays of urine, blood and hypophysis and in experimental parabiosis of intact with castrated or roentgen-rayed animals (20, 21, 23, 28, 54). Castrated female mice carrying intrasplenic ovarian transplants, but with vascularized adhesions that permitted ovarian hormones to bypass the hepatic portal circulation, showed estrous vaginal smears throughout the course of the experiment and acquired no ovarian tumors. Ovarian tumors have not occurred in the intrasplenic transplants of ovaries in unilaterally gonadectomized male and female mice (unpublished data). The only female mouse that had a granulosa cell tumor-like growth in the subcutaneously transplanted ovary was operated upon in pregnancy

and no estrous cycles were noted during the early part of the experimental period. All the other castrated male and female mice bearing ovarian transplants at sites other than those drained by the hepatic portal system, *e.g.*, subcutaneous or intratesticular grafts, had no ovarian tumors. It is not improbable that some of these grafts in the castrated mice might have become tumorous had they been observed at later dates.

It is of special interest that unmixed granulosa cell tumors developed in male, and luteomas in female, mice carrying the intrasplenic ovarian transplants. This may be due to the physiological difference in the pituitaries of males and females; such differences have been observed with rats (13, 66). The pituitary gland of males apparently produces less luteinizing hormone than that of the females. The luteinization of tumor cells and the transformation of the granulosa-like cells into luteomas indicate that the neoplastic growths are under the influence of the pituitary gonadotrophic hormones.

It seems possible that roentgen ray treatment of the ovaries in rats and mice may induce a similar endocrine imbalance. Levine and Witschi (49) reported that normal female rats showed constant estrus when placed in parabiosis with irradiated female rats, and the castration-cells were found in the pituitaries of the irradiated rats. Experimental induction of ovarian tumors in mice subsequent to roentgen ray irradiation has been reported by several workers. Because the histological structure of granulosa-cell tumors and the luteomas appearing in the ovaries grafted into the spleen are similar, if not identical, to the ovarian tumors induced with roentgen rays[3], it seems that the same mechanism, namely gonadotrophic overaction, is responsible for the tumors under both circumstances.

In the present experiments ovarian tumors occurred in mice of the A strain. The question of strain limitation in formation of the tumors in mice may be raised. Results of experiments in progress show rapid growth of the intrasplenic ovarian transplants in mice of several strains. Lipschütz and his collaborators (52) mentioned that species differences and the duration of the experiment may account for failure to obtain malignant ovarian growths in guinea pigs. Only a few of their animals carried intrasplenic ovarian grafts for more than 1 year. However, most of the ovarian tumors in the experiments with mice developed 7 months after the transplantation.

[3]Dr. H. S. Kaplan was kind enough to permit examination of some of his unpublished material of roentgen-ray induced ovarian tumors in mice.

One testicular tumor "bearing a striking resemblance to the granulosa-cell tumor of the ovary" and with certain portions showing arrhenoblastoma-like structures developed in an intrasplenic testicular transplant in a castrated male rat (5). Intrasplenic testicular transplants in castrated male and female mice have not become tumorous (51). The higher temperature in the abdominal cavity may prevent the development of testicular transplants (59).

II. Histogenesis of the Ovarian Tumors

Although the development of granulosa-cell tumors and luteomas has been studied by many investigators, the histogenesis of these tumors remains uncertain. The main controversial opinions on the histogenesis of granulosa-cell tumors reflect to a great extent the conflicting concepts of the development of definitive germ cells in the ovary. An adequate study of the histogenesis of the ovarian tumors has often been difficult because the comparatively large size of these growths precluded a systematic study of serial sections and frequently only late stages were obtained.

The histogenesis of granulosa-cell tumors has been explained by three major theories: (A) origin from differentiated granulosa cells, (B) origin from embryonic rests, and (C) origin from mesenchyme. Histological study of the present material suggests another view of the genesis of ovarian tumors, namely, that the tumors arise from the proliferation and ingrowth of germinal epithelium. The three theories may be briefly summarized as follows:

(A) Origin from differentiated granulosa cells. Schröder (76) designated the term "folliculoma" for some granulosa-cell tumors and stated that the tumors arose from the follicular epithelium. Robinson (72) reported that ova were present in the folliculoid spaces, and that the follicle cells could undergo structural and quantitative variations of the widest range and could give rise to benign and malignant tumors. He contended that the folliculoid structures in the tumors were not the result of a process of "liquefaction" or of "degeneration", not even of a possible "reversion to type" by the granulosa cells, but were the remains of ovarian follicles. Moreover, he believed that in man there is no postnatal ovogenesis from the surface epithelium of the ovary. Dockerty and MacCarty (16) described one granulosa-cell neoplasm and supported the view that the tumor arose from the follicle cells. It has been reported that in mice roentgen irradiation destroyed ova, but left the epithelium of small follicles intact (12, 29, 85) and that granulosa cell tumors originated from a proliferation of the persisting follicle cells. Others

have maintained that the ovarian tumors produced in irradiated mice were derived from the undifferentiated parenchymal cells of the ovary, and that the granulosa cells of the graafian follicle played no role (34). They observed, however, that the theca interna cells might participate with the parenchymal cells in the early postirradiation proliferation and luteinization.

(B) Origin from embryonic rests. According to Meyer (57, 58) the tumor cells originate from redundant granulosa cells that dip down into the ovarian substance from the germinal epithelium during the formative period and remain undifferentiated. These embryonic rests under certain unknown conditions become granulosa-cell tumors. Meyer based his theory upon an isolated finding of small masses of granulosa-cells in the ovary of a 45 year old woman, and assumed that such cells were situated chiefly in the medullary portion of the ovary. He contended that granulosa cell tumors cannot arise from epithelial cells of the true follicle because the life of the well differentiated granulosa cells is dependent upon living ova, and the majority of the tumors occur in old women in whom the ovaries no longer contain follicles. Te Linde (84) described one small granulosa-cell tumor located in the medulla near the hilus of the ovary, and assumed that the only possible origin of the tumor was an embryonic granulosa rest. Similar opinions have been expressed by others (32, 62).

(C) Origin from mesenchyme. Fischel (27) suggested that the ovarian stroma and follicular cells are derived from the ovarian mesenchyme, and the primitive mesenchymal cells could assume epithelial properties under the influence of the germ cells. He stated that the germ cells migrate into the gonad from the primordial gut during early fetal life, and the germinal epithelium, therefore, did not give rise to ova, follicle cells, or connective tissue, but existed throughout life merely as the peritoneal covering of the ovary. According to Fischel's theory, tumor cells of both granulosa and theca types arise through metamorphosis of the ovarian mesenchyme. Schiller (73, 74) supported this theory and stated that the transformation of granulosa-cell tumors from an immature trabecular "form" into a mature folliculoid "form" resulted from the transformation of connective tissue-like cells into the epithelial tumor cells. This mesenchymal theory has been used as a working hypothesis by several workers (37, 63, 64, 89). A participation in the histogenesis of granulosa-cell tumors also has been suggested (87).

The present experiments provided evidences that the germinal epithelium of the engrafted mouse's ovary proliferates and gives rise to ova and follicle cells to form primary ovarian follicles. The proliferation and ingrowth of the germinal epithelium were observed in all ovarian transplants that developed into tumors and in many of the nontumorous grafts in the intrasplenic, intratesticular, and subcutaneous sites. Local proliferation of the germinal epithelium with evidence of mitotic activity occurred frequently in the tumors. The intermingling of the invaginated epithelial cells with the cells of the smaller granulosa-cell tumors and the apparent transformation of the epithelial cells, with ellipsoidal nuclei and scanty cytoplasm, into the tumor cells indicated the genesis of granulosa-cell tumors from the germinal epithelium.

The ingrowths of germinal epithelium formed groups or networks of tubules or cysts. In some instances nuclei of the epithelial cells that lined the tubules appeared to become vesicular and the whole mass of epithelial ingrowth resembled the folliculoid formations. More often, however, the folliculoid structure seemed to form within small masses of diffuse tumor cells by subsequent liquefaction of some of the centrally placed cells. Schiller (73, 74) appeared to have noted transformations of this type although he did not associate the tumor formation with the activity of the germinal epithelium in his materials. Many of the follicle-like structures contained a few degenerating cells and sometimes blood cells or coagulated fluid, but no ovocytes were found in the folliculoid spaces in the present materials. Small ovarian follicles were rarely encountered at the periphery of ovarian transplants that developed into tumors. In the nontumorous ovarian transplants the number of ovarian follicles was generally decreased in proportion to the duration of the graft. There was no indication that the granulosa tumor cells were derived from the well differentiated granulosa or theca cells of the persisting ovarian follicles.

The observations made in the present study, as well as the histological examinations of ovaries of mice in other experiments in our laboratories, have not revealed embryonic rests of granulosa cells in the ovaries. Our experimental results do not support the assumption that such an embryonic rest of granulosa cells is a prerequisite condition for tumor formation. The tumors did not appear to develop in the medullary portion of the engrafted ovary but arose in conjunction with the germinal epithelium or in conjunction with ingrowths from the germinal epithelium. The proliferation and ingrowth of the germinal epithelium were not restricted to any particular region of the ovary.

Despite the discrepancy regarding the histo-

genesis of granulosa-cell tumors, pathologists agree that some ovarian tumors, *e.g.*, cystadenomas and adenocarcinomas, are derived from the germinal epithelium (32, 56, 72). The ingrowth of the germinal epithelium in roentgen-rayed ovaries of immature and mature mice, and rats has been described (18, 61, 66). Other workers (12, 29, 85) observed "tubular adenomas," but did not report a relationship between the invaginated epithelial cells and the granulosa-cell tumors and luteomas. However, the so-called tubular adenomas in roentgen-rayed ovaries of mice differ from tubular adenomas in the ovaries of old rats (22).

The fundamental issue in the histogenesis of granulosa-cell tumors is more closely associated with the development of ovarian follicle cells than the germ cells, although they have an intimate relationship. Allen (1) called attention to the cyclical production of new germ cells from the germinal epithelium during each estrous cycle in the adult female mouse. This observation on rhythmic activity of the germinal epithelium in mature female mice has been substantiated by several other workers (8, 9). Recently, Long (53) reported the growth *in vitro* of ovarian germinal epithelium from the adult mouse's ovary and observed the formation of primary follicles from the epithelial cells. The origin of definitive ova from the germinal epithelium in the ovaries of adult rats has also been studied (11, 24, 39, 48). Hargitt (39) described the production of ova, follicle cells, and interstitial cells from the germinal epithelium in adult rats' ovaries, and in old and senile rats the germinal epithelium continues to be active, especially in producing invaginated cords of cells, but frequently they are anovular. In an extensive investigation, Evans and Swezy (24) found that ovogenesis in the rat, guinea pig, dog, cat and man occurs throughout the whole period of sexual life in a rhythm fundamentally related to the ovulation cycle. The ova and follicle cells arise by proliferations from the germinal epithelium in the form of invaginations or cords. Hartman (41) and Everett (25) reported that in the adult opossum the germinal epithelium near the region of the hilus is more active in producing new ova; and Papanicolaou (65) suggested the increased activity in the hilus of the guinea pig's ovary was due to a greater supply of blood. Early studies on the continual proliferation of germ cells from the germinal epithelium have been reviewed (83). There are many reports supporting the view that the germinal epithelium is active in producing ova in adult mammalian ovaries: the bat (38), the guinea pig (7, 40, 75), the rabbit (19), the ground squirrel (70), the prairie dog (82) and the dog (2).

Luteomas appear to arise also from the tubular ingrowths of the germinal epithelium, although there are instances showing that the tumors may arise indirectly by transformation from granulosa tumor cells. The cells in a granulosa-cell tumor may partially luteinize, and large lutein cells are present among the luteomatous cells. The possibility that luteomas originate from proliferation of the lutein cells, as suggested by Butterworth (12), was not confirmed by our observations. Luteinization of the granulosa tumor cells has been reported (43, 63, 86). The present studies reveal that granulosa-cell tumors and luteomas are derived from the germinal epithelium. The general concept of ovarian epithelial tumors originated from the invading germinal epithelium has been postulated by Goodall (36) and others.

SUMMARY AND CONCLUSIONS

1. Five granulosa-cell tumors, 2 pretumorous lesions and 1 mixed tumor (granulosa and luteoma cells) were found among 21 castrated male mice of the A strain bearing intrasplenic ovarian transplants. Five luteomas and 7 mixed tumors occurred in intrasplenic ovarian transplants in 52 castrated female mice of the same strain. Most of the ovarian tumors developed after 7 months from time of operation.

2. Castrated female mice bearing intrasplenic ovarian transplants with vascularized adhesions that permitted drainage through other than the hepatic portal system showed estrous vaginal smears throughout the course of the experiment and acquired no ovarian tumors. The only female mouse that had a granulosa cell tumor-like growth in the subcutaneously transplanted ovary was operated upon during pregnancy and no estrous cycles were noted during the early part of the experimental period. All the other intact or castrated male and female mice bearing ovarian transplants in subcutaneous or testicular sites had no ovarian tumors.

3. The intrasplenic ovarian transplants in the gonadectomized hosts attained larger size and more frequently showed extensive tubular ingrowth of germinal epithelium than did those at other sites.

4. The germinal epithelium in the ovarian transplants give rise to the primary ovocytes and follicle cells. The granulosa-cell tumors appear to be derived from the ingrowth of germinal epithelial cells.

5. The luteomas develop either directly from the ingrowth of the germinal epithelium or indirectly from granulosa-cell tumors.

6. Ovarian hormones produced by the intrasplenic ovarian transplants are inactivated by the liver before entering the systemic circulation, while the hypophysis, under such conditions as physiological castration, produces larger amounts of gonadotrophic hormones to further stimulate the ovarian transplants. The endocrine imbalance so established is assumed to be responsible for the genesis of the ovarian tumors in the present experiments and possibly also in the ovaries of roentgen ray irradiated mice.

7. The influence of ovarian transplants on the accessory genital tissues and other organs in mice was described.

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Dosage of Carcinogen as a Modifying Factor in Evaluating Experimental Procedures Expected to Influence Formation of Skin Tumors*

Albert Tannenbaum, M. D., and Herbert Silverstone

(*From the Department of Cancer Research,** Michael Reese Hospital, Chicago 16, Illinois*)

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Although a particular experimental procedure might augment or inhibit tumor incidence when the carcinogenic stimulus employed to induce tumors is mild or moderate, it is possible that the effect might not be evident when higher dosages of carcinogen are utilized. This possibility was suggested by negative results of investigations in which only large dosages of carcinogen were employed, and by a few studies in which the effects of both small and large dosages of carcinogen were compared. At a meeting at Madison, Wisconsin, in 1937, Kreyberg referred to massive amounts of carcinogen as "steam-roller" doses, implying that they were large enough to mask or obliterate the effects obtainable by the same experimental procedure but with smaller doses of carcinogen.

In evaluating carcinogenesis, at least two aspects of tumor formation may be considered: (a) the incidence of tumors, and (b) the time of appearance of the tumors. Although a large dose of carcinogen may induce up to 100 per cent tumors in both a control and experimental group of animals, it is still possible that differences in the time of appearance of the tumors may be demonstrated.

In experiments reported from this laboratory it was shown that calorie-restricted diets inhibited the formation of benzpyrene-induced skin tumors in mice (9) and that fat-enriched diets (10) enhanced the formation of such tumors. In those experiments the skin tumors were induced by application of moderate dosages of carcinogen. The tumor response was controlled by terminating the application of carcinogen before any appreciable number of skin tumors had appeared, the dosage being gauged to produce tumors in between 40 and 70 per cent of the mice in the control (*ad-libitum*, low-fat diet) groups. The influence of calorie restriction or fat enrichment of the diet was demonstrated principally by the effect on the incidence, or number of mice developing tumors. Only secondary attention was given the rate of appearance of the tumors, although it was pointed out that in those groups in which fewer tumors arose, these tumors tended to appear, on the average, at a later time.

This report is concerned with experiments designed to test whether or not the inhibiting effect of calorie restriction and the augmenting effect of fat enrichment of the diet, observed when moderate dosages of carcinogen were utilized, would be obliterated by the use of larger amounts of carcinogen.

METHODS

In both of the studies reported here 4 groups, each of about 50 dba strain male mice, were utilized. The mice were born within a 4 week period and at weaning the litter mates were distributed between the 4 groups of the experiment. When the mice were about 9 weeks of age they were transferred from the stock diet of Purina dog chow checkers to their respective experimental diets.

In each study, 2 of the 4 groups were fed a control ration consisting of 1.4 parts of Purina dog chow meal, 0.9 parts of skimmed milk powder, and 1.9 parts of cornstarch. This ration permits mice to grow normally. It contains 15 per cent protein, 2 per cent fat, and 70 per cent carbohydrate, along with adequate vitamins and minerals. The other 2 groups of the particular study were fed the experimental diets (calorie-restricted or fat-enriched) which are described later in the experimental sections.

Application of carcinogen (one drop of a 0.3 per cent solution of 3,4 benzpyrene in benzene, applied by means of a dropper to the skin of the interscapular area) was begun 4 weeks after the diets had been initiated. In each study the mice of 1 control group and of 1 experimental group were given 19 applications in 9 weeks, while the mice of the other control group and experimental group were given 50 applications in 18 weeks. Thus the former 2 groups were considered to have received

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a moderate dosage of carcinogen, and the latter 2 groups a large dosage. This assumption was confirmed by the tumor response.

The housing, feeding, care, inspection, and observation of the animals have been described in previous publications (9, 10). The animals were examined for skin tumors and weighed in general, at 2 week intervals except during the period when the tumors were appearing at a rapid rate; during this time the mice were inspected once a week. The percentage of mice with tumors was calculated on the basis of the number of animals alive at the time the first tumor appeared in the experiment (effective total). The total number of mice with tumors and time of appearance of tumors refer to the first tumor appearing in each mouse. In most mice the tumors were recognized initially as papillomas, in others as carcinomas; these were all grouped and reported as skin tumors.

EXPERIMENTAL

The Effect of Dosage of Carcinogen on the Inhibiting Action of a Calorie-Restricted Diet on the Formation of Induced Skin Tumors

In this experiment the 2 groups (A0, A60) that received the control ration (as described in Methods) were given 4.2 gm. per mouse daily. The mice of the 2 groups given the calorie-restricted ration (A5, A65) were given 2.3 gm. of a ration consisting of the same amount of Purina dog chow meal and skimmed milk powder, but without the added cornstarch. The control mice ate, on the average, 3.8 to 4.1 gm. daily, while the calorie-restricted mice ate all of the food given them, namely 2.3 gm. One may consider that the mice of all 4 groups consumed relatively equal quantities of protein, vitamins, and minerals and that the mice on the restricted diet were restricted in carbohydrate only.

Control group A0 and calorie-restricted group A5 received the moderate dose of carcinogen while control group A60 and calorie-restricted group A65 were given the large dose of carcinogen. At the initiation of the experimental diets, 4 weeks before the first application of carcinogen, the mice of each of the 4 groups weighed 22 gm., on the average. From the beginning of the application of carcinogen until the end of the experiment, the mice of the calorie-restricted groups maintained an average weight of 21 gm. Mice of the *ad-libitum* control groups had grown to 27 gm. on the average when the application of carcinogen was begun. During the following 16 weeks the control mice receiving the moderate dosage (A0) grew to an average weight of 34 gm. and very nearly maintained this weight during the subsequent course of the experiment, while the mice given the large dosage (A60) grew somewhat more slowly reaching only 31 gm. average weight (due probably to the greater toxicity of the larger dosage of carcinogen).

Groups A60 and A65 (large dosage) were terminated at 38 weeks after the first application, at which time none of the A60 mice were alive and no new tumors had appeared in A65 for 8 weeks. Groups A0 and A5 (moderate dosage) were terminated at 60 weeks since by this time very few tumors were appearing. The curves of tumor formation are presented in Fig. 1 and salient data and statistics in Table I.

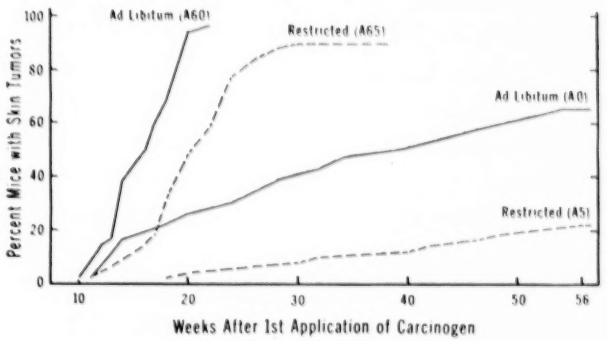

FIG. 1.—Formation of skin tumors induced by 3, 4-benzpyrene. Curves showing effect of caloric restriction when moderate dosage (A0, A5) and large dosage (A60, A65) of carcinogen where utilized.

Effect on incidence of tumors.—The striking effect of caloric restriction on the incidence of mice developing skin tumors observed when the dosage of carcinogen was moderate (65 per cent in the control A0, as compared with 22 per cent in the calorie-restricted group A5) was obliterated by the higher dosage (96 per cent in the control A60, and 90 per cent in the calorie-restricted group A65). However, a residuum of the effect on incidence is suggested by the fact that 3 of the mice of the calorie-restricted group, A65, had not developed skin tumors 38 weeks after the first application of carcinogen—8 weeks after the last tumor had formed in this group and 16 weeks after all the mice of the *ad libitum* control group, A60, had either developed skin tumors or died.

Effect on time of appearance of tumors.—The use of the high dosage of carcinogen yielded results which emphasized the effect of calorie-restriction on the mean time of appearance of tumors. While the mean times of appearance were shortened, and even the actual magnitude of the difference was decreased by the employment of a large dosage of carcinogen, the difference has greater statistical significance because the range of time over which

the tumors appeared was markedly decreased. The question of statistical significance as related to evaluating the effects of caloric restriction will be considered more fully in the discussion.

The data suggest that with moderate dosages of carcinogen the effect of caloric restriction on tumor formation is revealed principally through a reduction in the incidence of tumors, while with large dosages of carcinogen principally through the delay or postponement in appearance of the tumors.

TABLE I: THE EFFECTS OF CALORIC RESTRICTION AS INFLUENCED BY DOSAGE OF CARCINOGEN

0.3% Benzpyrene in benzene	Group	Number of mice	Mice alive and free of tumors at end of experiment	Mice with tumors		Time of appearance of skin tumors (weeks)				
				No.	%	Mean	Variance*	Difference between means	Significance* of difference t	Significance* of difference P
Moderate dosage, 19 applications	A0: Adlibitum	49	4	32	65	28.3 ± 2.4	178	9.7 ± 4.6	2.1	less than 5%
	A5: Caloric restricted	50	26	11	22	38.0 ± 4.0	172			
Large dosage, 50 applications	A60: Adlibitum	49	0	47	96	16.3 ± 0.5	10.3	4.4 ± 0.81	5.5	less than 0.1%
	A65: Caloric restricted	48	3	43	90	20.7 ± 0.7	18.3			

*In Tables I and II, the "variance" is a measure of the dispersion of the times of appearance (the square root of the variance is the standard deviation). The greater the tendency for the individual values to gather about the mean time of appearance, the smaller the variance. "t" is the ratio of the difference between the two means being compared to an estimate of the standard error of the difference; the standard error being a function of the variances of the two groups. "P" is the probability that the value of "t" could have occurred on the basis of chance alone—*i.e.* if there had been no real difference between the groups due to the experimental procedure. It is obtained from prepared Tables which give the relation between "t" and "P." The greater is t, the smaller is P and hence the less the probability that the observed difference is not real. A value of P less than 5% is generally considered to indicate "statistical significance" of the difference. These terms and their implications are considered further in the discussion; details of the test and its application may be found in any recent standard text on statistics (4).

EFFECT OF DOSAGE OF CARCINOGEN ON THE AUGMENTING ACTION OF A FAT-ENRICHED DIET ON THE FORMATION OF INDUCED SKIN TUMORS

The two groups (S10, S60) that received the control ration (as described in METHODS) were given 4.2 gm. per mouse daily. The mice of the 2 groups (S11, S61) fed the fat-enriched diet were given a ration containing the same amount of Purina dog chow meal and skimmed milk powder, but 0.9 gm. of fat[1] were substituted for 1.9 gm. of cornstarch. The resultant ration contained 19 per cent protein, 31 per cent fat, and 38 per cent carbohydrate. The control mice ate, on the average, 3.8 to 4.2 gm. daily, while the mice on the fat-enriched diet ate from 2.9 gm. to all of the 3.2 gm. given them. For practical purposes one may consider that the mice of all 4 groups ate approximately equal amounts of protein, vitamins, and minerals.

Control group S10 and fat-enriched group S11 received the moderate dose of carcinogen while the control group S60 and the fat-enriched group S61 were given the large dose of carcinogen.

At the first application of carcinogen, the average weight of the mice of each group was 25 gm. During the following 16 weeks the mice of the groups receiving the moderate dosage (S10 and S11) grew to an average of 32 gm. and very nearly maintained this level during the course of the experiment. The increased toxicity of the large dosage was manifested in the slightly depressed growth of the mice given 50 applications of carcinogen (S60 and S61); the mice of these 2 groups reached a maximum average weight of 30 gm.

The experimental groups receiving the large amount of carcinogen (S60, S61) were terminated at 31 weeks after the first application of carcinogen, and those given the moderate dosage (S10, S11) were terminated at 56 weeks, since there were very few mice without tumors at these times. The curves of tumor formation are presented in Fig. 2 and summarized data are given in Table II.

Effect on incidence of tumors.—In the two groups to which the moderate dosage of carcinogen was applied, the incidence of mice with tumors was 68 per cent in the control S10 group compared with 78 per cent in the S11 group consuming the fat-enriched diet. This difference, while small and not in itself of statistical significance, is reproducible (10) having been obtained repeatedly in our laboratory when dosages of comparable order were employed.

On the other hand, the application of the larger amount of carcinogen obliterated the slight augmentation of incidence of tumors, 96 per cent and 98 per cent occurring in groups S60 and S61 respectively.

[1]Kremit—partially hydrogenated cottonseed oil, furnished generously by Armour and Company, Chicago.

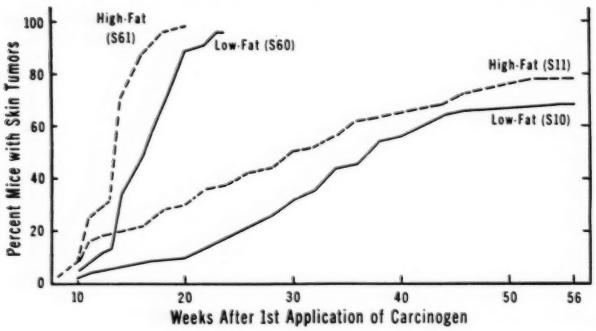

FIG. 2.—Formation of skin tumors induced by 3, 4-benzpyrene. Curves showing effect of a fat enriched diet when moderate dosage (S10, S11) and large dosage (S60, S61) of carcinogen where utilized.

Effect on time of appearance of tumors.—The difference in mean time of appearance of tumors, between groups S10 and S11 (moderate amount of carcinogen) was not of statistically significant magnitude, although it is in the direction expected from similar experiments previously reported by us. In the group developing more tumors the growths occurred at an earlier average time.

More definitively, when using the larger amounts of carcinogen, the group given the high-fat diet (S61) developed tumors at a significantly (statistically) earlier time than the group consuming the control ration. This difference in time of appearance, 2.8 weeks, is significant even though relatively small. The range of time of appearance of the tumors was abbreviated by use of the large dosage of carcinogen to the extent that even a difference of about one-half that actually obtained would have statistical significance.

This latter point is of interest since in no single previous experiment on the effect of a fat-enriched diet on skin tumor formation have we obtained a difference of statistically significant order (and hence acceptable as reliable evidence). Existence of this so called "fat-effect" was accepted because of the reproducibility of the augmenting effect, and because of the comparable results of other workers (5, 11). This experiment (S60 and S61) is therefore the first single experiment with induced skin tumors, in which using groups of only 50 mice we have obtained a difference that in itself, on the basis of statistical criteria and independent of the results of other experiments, proves the augmenting effect of a fat-enriched diet.

The evaluation of these results (effect of fat-enriched diet) is in agreement with that of the previous experiment (effect of calorie-restricted diet). With moderate dosages of carcinogen these dietary changes revealed their effects on the formation of induced skin tumors principally through changes in incidence, while with large dosages the effects were shown mainly through changes in the mean time of appearance (induction time).

DISCUSSION

In the present experiments, the large dosage of carcinogen, by producing a very high percentage of tumors in both control and experimental groups, obliterated the differences in *incidence* of tumors. On the other hand, the large dosage of carcinogen actually emphasized the differences in *time of appearance* of tumors. The ability to demonstrate this depended on greater statistical validity rather than on increases in the magnitude of the differences. The increased statistical significance arose from the fact that in the mice treated with the large dosage, the tumors formed within a very much shorter span of time than did the tumors of the mice treated with the smaller amount of carcinogen. The interpretation will be accepted by those with an understanding of statistics; for others, a brief discussion of the principles involved is given. This is followed by a general discussion.

Statistical.—Throughout this report the term "significance" is employed only in the statistical sense—*i.e.* it is not necessarily related to the biological importance of the effects observed. Most statistical "tests of significance" actually test the

TABLE II: THE EFFECTS OF A FAT-ENRICHED DIET AS INFLUENCED BY DOSAGE OF CARCINOGEN

0.3% Benzpyrene in benzene	Group	Number of mice	Mice alive and free of tumors at end of experiment	Mice with tumors		Time of appearance of skin tumors (weeks)				
				No.	%	Mean	Variance*	Difference between means	Significance* of difference t	Significance* of difference P
Moderate dosage, 19 applications	S10: Low fat	50	3	34	68	31.4±1.8	105	4.6±2.8	1.6	greater than 5%
	S11: Fat-enriched	50	7	39	78	26.8±2.1	176			
Large dosage, 50 applications	S60: Low fat	45	1	43	96	16.6±0.5	11.4	2.8±0.63	4.4	less than 0.1%
	S61: Fat-enriched	48	0	47	98	13.8±0.4	6.0			

*See footnote, Table I.

hypothesis that there is no real difference between the groups being compared (no effect of the experimental procedure)—that the difference observed is no greater than could be expected on the basis of "chance" alone. The validity of this hypothesis is measured by the probability, P; the smaller is P, the less the probability that the procedure was without effect. Obviously, the level at which this probability will be considered to imply significance is arbitrary, but most workers accept the conclusion of statistical significance if (a) P is less than 5 per cent, (b) the experiment proceeded "satisfactorily" with respect to the controlled and measured aspects, (c) all uncontrolled factors may be considered to have affected the groups comparably, and (d) the resulting conclusions are rational in the light of general experience and concepts.

In the present investigations, one may evaluate the effects of the experimental procedure by considering (a) the incidence of tumors—*i.e.* the percentages of mice developing tumors (by a specific time or by the end of the experiment), and (b) the time of appearance of tumors (induction time, latent period, etc.) as measured from some arbitrarily selected moment, such as that of the first application of carcinogen.

The statistical significance of the difference in *incidence of tumors*, may be measured in any of several ways, for example by the chi-square test (4). Employing these tests, the incidence of tumors in A5 was significantly less than in A0, ($\chi^2 = 18.9$, P less than 0.1 per cent) while the other pairs, A60 v A65, S60 v S61, or S10 v S11 did not differ significantly.

In order to determine statistical significance of the differences between the several pairs in *times of appearance* of skin tumors the t-test was employed (4). In this test the significance of the difference between "times of appearance" is determined from the following ratio: $t = \frac{M_1 - M_2}{S_d}$,

where M_1 and M_2 are the "mean" times of appearance of tumors in the two groups being compared, and S_d is the "standard error" of the difference; S_d is proportional to the variances (or "scatter") of the times of appearance of tumors in the two groups. Greater values of "t" are associated with decreased probabilities (P) that the difference could be due to "chance." Obviously then, if the standard error of the difference decreases, as the variances of the two groups decrease, there will be a tendency toward an increased value of "t" and to increased "significance" of the difference.

Bryan and Shimkin (2) have shown in studies on sarcoma-formation that, within certain limits, increased dosage of carcinogen effects a reduction in both the mean and the variance of the times of appearance of tumors. Their data indicate that, as a consequence of this association between dosage and variance, increased dosage of carcinogen leads, for groups of equal size, to increased "reliability" of the data on times of appearance. With reference to the experiments reported here, the action of the increased dosage of carcinogen caused not only a definite decrease in the mean time of appearance of tumors but also a considerably greater decrease in the variances (A0 v A60, A5 v A65 etc.). In consequence, while the actual *magnitude* of the difference between the groups being compared was decreased by the greater carcinogen dosage (*e.g.* compare the difference between the mean times of appearance of S10 and S11 with the difference between S60 and S61) the standard error of the difference was reduced to a much greater extent. As a consequence, the values of "t" are greater (and P is smaller) for the comparisons between the groups given the greater dosages; and in these groups there was much less possibility that the differences were due to "chance."

Those familiar with the limitations of the t-test may be interested in the following considerations. One condition of the t-test is that the distributions of the times of appearance of tumors in each group do not deviate greatly from the "normal" distribution. In the present experiments none of the groups revealed statistically significant deviations from the normal distribution. Secondly, the derivation of the t-test requires that the variances of the two groups being compared should not differ significantly if the test is to afford accurate measure of the significance of the differences between the *means*. However, regardless of differences between variances, the test does measure the significance of the difference between the *distribution* of times of appearance. For example, if a value of "t" corresponds to a significantly low P, it seems relatively unimportant from a practical viewpoint whether the treatment produced only a change in the means, or in the variance also; the point is, the experimental procedure has produced a difference of statistically significant order in the times of appearance. In applying the test to the data given in Tables I and II, it was recognized that the variance of S60 was significantly greater than that of S61, and that of A65 greater than that of A60. A device commonly used to "normalize" distribution and to minimize differences in variance is the transformation of "time" to some other function such as "logarithm of time." This transformation, applied to the present data, gave some-

what better approximation to the normal distribution, and better agreement between the variances of the groups compared. The results of the logarithm transformation are given in Table III.

Regardless of the basis of comparison (time or logarithm of time) it is obvious that despite application of "large" dosages of carcinogen, the delaying effect of caloric restriction and the accelerating effect of a fat-enriched diet on the time of appearance of tumors are demonstrable. Moreover, it is apparent that employment of the larger dosage increased the "statistical significance" of these differences. However, since there is probably a minimal time before which tumors may not arise despite increased dosages of carcinogen (2), it is possible that extremely large dosages might even obliterate differences in the times of appearance.

TABLE III: RESULTS OF LOGARITHMIC TRANSFORMATION OF THE DATA ON TIMES OF APPEARANCE OF SKIN TUMORS

Dosage of carcinogen	Group	Logarithm of the time of appearance of skin tumors (log. weeks) Mean	Variance	Difference between means	Significance of difference t	P
Moderate	A0: *Ad libitum*	1.403	0.0445	0.150 ± 0.063	2.3	less than 5%
	A5: Calorie-restricted	1.553	0.0280			
Large	A60: *Ad libitum*	1.203	0.00801	0.104 ± 0.019	5.4	less than 0.1%
	A65: Calorie-restricted	1.307	0.00895			
Moderate	S10: Low fat	1.470	0.0285	0.100 ± 0.048	2.1	less than 5%
	S11: Fat-enriched	1.370	0.0558			
Large	S60: Low fat	1.211	0.00823	0.078 ± 0.018	4.3	less than 0.1%
	S61: Fat-enriched	1.133	0.00642			

General.—In reference to the effect of dosage of carcinogen on the incidence of tumors two additional aspects deserve mention. In the experiment concerned with caloric restriction, the larger dosage of carcinogen practically eliminated the difference in incidence obtained with the smaller dosage. The data were presented without any distinction as to whether the tumors were papillomas or carcinomas, although the exact types of tumors and their progress were observed and noted in the records of the experiment. These reveal that somewhat more of the mice of the A60 group developed carcinomas than those of the A65 group.

The character and development of the tumors formed in the various groups followed, in general, a consistent pattern. In the lower dosage groups (A0, A5, S10, S11) single papillomas formed, and these were clearly demarcated from the rest of the skin to which the carcinogen had been applied. In contrast, the mice of the groups (A60, S60, S61, but not A65) given the larger amount of carcinogen developed 2 to 5 papillomas in the treated area; these often became confluent. As the lesions became carcinomatous differences between the low and high dosage were still apparent. The single discrete papillomas in those given the smaller dosage revealed a firm "pearly" periphery as the base, and the palisade structure of the papilloma was replaced by a horny, necrotic, or hemorrhagic crust. On the other hand, the papillomas of mice given larger dosage of carcinogen were converted into carcinomas with a broader base, on the surface of which the papillomas persisted for a longer time. By the end of the experiment the differences between the groups treated with the moderate and large amounts of carcinogen were less striking, but still apparent. The inhibiting effect of caloric restriction on tumor formation was reflected in the fact that the mice of the A65 group (higher dosage, calorie-restricted) presented tumor characteristics more like those of the mice on lower dosage, rather than those of the mice of the other higher dosage groups, A60, S60, S61.

That the relative effect of the dietary procedure may be independent of the dosage of carcinogen is shown by a comparison of the times of appearance of tumors in the groups (Table IV). At both dosages of carcinogen the calorie-restricted diet prolonged the mean time of appearance by about 30 per cent; the high fat diet shortened the mean time of appearance by about 16 per cent.

For various types of tumors it has been shown that with increasing carcinogenic stimulation the incidence of tumors increases until nearly all of the treated animals develop tumors. In general, the

increasing dosage also shortens the average time of appearance (induction time) of the tumors. The correlation, between incidence and mean time of appearance of tumors, is evident not only from investigations in which the carcinogenic stimulus was the sole variant but also from studies in which the carcinogenic stimulus was constant, the variant being an experimental procedure. Loeb (7), in reference to spontaneous mammary carcinoma in different strains of mice, observed "that between the frequency of cancer and the average age at which it appears a quantitative relation existed in the sense that, on the whole, the greater the frequency of cancer the earlier the appearance." Similarly, with regard to the many investigations performed in this laboratory on the effect of dietary changes on the formation of spontaneous and induced tumors, we have noted that the tumors in the group (control or experimental) in which the greater number occurred usually had a shorter mean time of appearance. There is a limit to the relationship, however, in that no more than 100 per cent tumors can be formed, and there is probably a minimal time before which tumors do not appear despite further increases in dose of carcinogen.

TABLE IV: RATIOS OF MEAN TIMES OF APPEARANCE OF SKIN TUMORS

Ratio	Dosage of carcinogen	
	Moderate	Large
of Calorie-restricted group to *Ad libitum* control group	1.34	1.27
of Fat-enriched group to Low fat control group	0.85	0.83

Loeb (7), as early as 1924, was of the opinion that if a stimulant was strong enough it could mask or overwhelm any inherited resistance so that all of the hosts develop cancer. Similarly, Watson and Mellanby (11) "anticipated that any resistance induced by a particular experimental treatment would be masked if the conditions for inducing the tumors were too severe." Accordingly, in experiments designed to test the modifying effect of diet or treatment of the skin on the formation of tumors, these authors utilized technics to avoid large dosages of carcinogen (tar) such as fewer paintings or applying the tar with the tip of the brush. Since then other investigators (1, 3, 10) have expressed the view that factors which influence tumor formation might have been overshadowed, obscured, or masked when large dosages of carcinogen were employed. Others have attributed failure to reveal effects of experimental procedures to the use of large dosages of carcinogen.

To our knowledge, the first experimental support of this concept was presented in the work of Shear and his co-workers (3, 8) on the promoting effect of a basic fraction of creosote oil. Leiter and Shear (6) have reported a large number of investigations concerned, in part, with the effect of sex on the production of subcutaneous tumors in mice. They found that the percentage of tumors was greater in the males, (although not invariably) and that the ratio (incidence in males: incidence in females) decreased with increasing level of tumor production.

These considerations are of interest in light of the results reported in this communication. In conformity with these findings, the high dosage of carcinogen tended to obliterate the differences between the control and experimental groups in incidence of tumors. On the other hand, differences between times of appearance of the tumors (induction time) were actually emphasized because of increased statistical validity. The high dosage of carcinogen masked the effect of two different experimental procedures on the incidence, but made more evident the effect on the rate of formation of the tumors. This suggests that employment of two criteria—incidence and induction time—or more, might yield better evaluation of the effect of an experimental procedure. In some instances the utilization of large dosages of carcinogen might actually be advantageous, particularly in demonstrating the effects of an experimental treatment on the time of appearance of tumors.

SUMMARY

There is a prevailing view that large dosages of carcinogen may obliterate the effect of an experimental procedure on the induction of tumors. This is reasonable since the incidence of tumors increases with increasing amounts of carcinogen, finally approaching the maximum of 100 per cent. Since, in general, increasing incidence of tumors is associated with the shortening of the mean time of appearance of these tumors, it seemed worthwhile to inquire into the effect of large dosages of carcinogen on the latter criterion of tumor formation.

The inhibiting effect of caloric restriction and the enhancing effect of a fat-enriched diet on the formation of induced skin tumors was investigated at two levels of carcinogen dosage, moderate and high. The results showed that large amounts of carcinogen tended to mask the action of the experimental procedures in regard to incidence of tumors. On the other hand, the effect on time of

appearance (induction time) of the tumors was actually emphasized through increased statistical validity.

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The Influence of Mammalian Environments on the Tissue Specificities of the Rous Chicken Sarcoma Virus*

Edward W. Shrigley, Ph. D., M. D.

(From the Department of Bacteriology and Immunology, Yale University School of Medicine, New Haven 11, Connecticut)

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Since the Rous chicken sarcoma virus undergoes variation in its tissue specificities following growth of the tumor in the anterior chamber of the guinea pig eye (6), it was considered of interest to determine whether other mammalian hosts have a similar influence on this agent. The Rous tumor, therefore, has been transplanted into the anterior chamber of the mouse eye in an effort to see first if growth of the tissue would take place, and second if the virus would undergo variation. The present communication gives in detail the results of these experiments, and compares them with those already reported for chicks inoculated with the guinea pig passage tumor.

MATERIALS AND METHODS

Avian hosts.—In all cases, chicks obtained from a local commercial hatchery were of the Plymouth Rock breed. These birds varied in age from 1 to 60 days at the time of injection. The different strains of Rous tumor were maintained in chicks after a single rodent passage, tissue specificities of the virus being observed in the avian hosts. Only those birds presenting one or more of the protean lesions of the Rous virus infection are included in the present data. All inoculated individuals, both experimental and controls, were kept together in the same brooder.

Mammalian hosts.—The guinea pigs used were obtained from the random bred stocks of local dealers, and varied in age from 6 to 8 months (6). On the other hand, the mice utilized for intraocular inoculations of the Rous tumor were young adults of the "A" inbred strain. A few individuals of the C3H stock also were inoculated, but observations were more difficult in the pigmented eye of the agouti than in the pink eye of the albino, and use of the former strain was abandoned. All of the data to be considered here deal with observations on tumors passed through "A" strain mice.

Tumor material.—The strain of Rous tumor used in the guinea pig experiments was obtained from Dr. F. Duran-Reynals. This sarcoma has been under study by him for the past 7 years. Mouse inoculations were carried out with a strain of the Rous sarcoma which was obtained in 1944 from Dr. Albert Claude of the Rockefeller Institute. Both tumor strains yield virulent virus which causes pronounced lesions of all gradations ranging from those predominantly hemorrhagic to those entirely neoplastic.

Since the differences in the incidence of involvement of the various chicken tissues by the two stock viruses did not vary beyond the limits of random sampling, the observations within these two groups of birds were pooled and used as controls.

Methods.—The technic employed for the transplantation of tumor tissue into the mouse eye is essentially that used for the guinea pig inoculation (6). Sarcomas for transfer were obtained from freshly killed birds. These growths had developed in the breast following an intramuscular injection of tumor filtrate (Berkefeld "N") 10 days to 2 weeks previously. For guinea pig inoculation the tumor tissue was divided into small pieces approximately 2 to 3 cu. mm. in size, and placed into a No. 2 trocar. The material for mouse injection was cut into considerably smaller portions since these were made to fit into a 22-gauge spinal needle (5). The anterior chamber of the rodent eye was opened by plunging the point of a Bard-Parker No. 11 blade through the cornea at the sclerocorneal junction. The fragment of tumor to be transplanted was inserted into the eye by means of the trocar or needle and forced to the lower portion of the chamber by means of the stilet plus slight pressure on the corneal surface.

The guinea pigs were killed 10 or 11 days after inoculation and the growths were transferred to the breast muscle of chicks by means of the trocar. The mice were sacrificed at various times from the first to the 29th day after injection and the transplants were inserted into avian hosts in a manner similar to the above. From these rodent passages, then, two lines of Rous tumors were established in chicks; the one coming from the guinea pig pas-

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sage is referred to in this paper as the "guinea pig passage tumor", and the other from the mouse, the "mouse passage tumor". The sarcoma strains that have never been inoculated into either rodent are referred to as "stock Rous tumor".

Following the transfer of the tumor from the rodent to the chick, subsequent inoculations were made into birds by either the intramuscular route into the breast (cell suspension diluted 1/5 by weight) or a combination of the intravenous route into the external jugular and intramuscular route into the breast with Berkefeld "N" filtrates (dilution 1/20 by weight). The data here presented have been obtained from observations on birds representing from 5 to 8 serial passages of guinea pig passage tumor, and on 5 avian transfers of the mouse passage material. It should be emphasized that the observations on the guinea pig passage tumor material represent a pooling and further analysis of data already presented (6) and stem from growths obtained from the inoculation of 3 different guinea pigs. On the other hand in the present experiments 23 mice yielded tumors that grew in chicks, and the data from the mouse passage material is obtained from pooled observations of these lines over 5 serial bird passages.

EXPERIMENTAL

Properties of the guinea pig passage tumor strain.—The chicks receiving the Rous tumor directly from the rodent did not show any unusual distribution of tissue involvement. Three of the 9 birds inoculated with this material developed hemorrhagic lesions, thus indicating that the virus had persisted throughout the residence of the transplant in the rodent eye. Further, 6 chicks had a tumor localized at the inoculation site in the breast and the other 3 showed an extension of the local lesion to the liver. There was no evidence of periosteal growths in these individuals of the first avian passage. Subsequent transfer of these tumors into chicks gave evidence that the tissue specificities of the virus had changed. While many new areas of the host were involved by the virus the development of periosteal tumors was considered to be the most consistent indication of virus alteration (2). Of 121 birds inoculated with Berkefeld "N" filtrates of this tumor strain, 43 per cent subsequently possessed one or more bone lesions. Evidence suggests that this alteration in tissue specificities of the virus may not be a transient affair. Throughout 8 chicken passages the frequencies of those with and without bone tumors did not vary beyond the limits of random sampling.[1] However, the total number of birds in the last 2 passages is small. The frequency of bone lesions in the sixth chicken passage is the most out of line (Table I).

TABLE I: INCIDENCE OF BONE LESIONS IN BIRDS OF SUBSEQUENT CHICKEN PASSAGES INOCULATED WITH FILTRATES OF THE GUINEA PIG STRAIN OF ROUS TUMOR

No. of birds with	No. of chicken passages 2	3	4	5	6	7	8	Total No. of birds	Probability†
Bone lesions	9	13	9	10	5	4	2	52	0.42
No bone lesions*	8	13	11	14	16	3	4	69	

*Those birds not showing bone lesions all had other manifestations of Rous virus infection.

†The probability that these two distributions are samples from the same population. Probabilities of 0.01 or less are the only values of "P" considered significant.

Other tissues of the chick not normally involved were also invaded by the altered agent, but not consistently enough to warrant statistical analysis. The most interesting of these was the occurrence of 2 ulcers in the mucosa of the gizzard (Fig. 1). Fig. 2 shows these lesions to be invasions and eventual replacements of the mucosa by the malignant fibroblasts, the tumor probably arising from the submucosal area.

Morphological evidence that the growths of the guinea pig passage strain metastasize by cell emboli in the blood stream may be seen in Fig. 3. Also these growths actually invade tissue cells (Fig. 4). The histology of this rodent tumor strain was essentially the same as that found in the stock Rous growths no matter whether the transplant was in the rodent or chick. The behavior of the chicken sarcoma in the guinea pig eye has been described (6).

Growth of the Rous chicken sarcoma in the mouse eye.—Of the 31 mice inoculated intraocularly with this sarcoma, 23 yielded growths resulting in tumors on injection into chicks. The criteria for growth of the transplant in the rodent were: (a) the increase in size of the transplanted tissue, (b) the presence of mitotic figures in the tumor, and (c) the ability of the transplant to produce growths in chicks. The second criterion was found not to be too reliable since mitoses were frequently difficult to find yet the other two criteria were easily fulfilled. Due to the size of the eye it was difficult to determine the onset of vascularization. The transplanted tissues were allowed to remain in the mouse eye for periods varying from 1 to 29 days.

[1] In the present study, probabilities as determined by chi-square tests of 0.01 or less are the only values of "P" considered significant.

During this time growth was manifest by an increase in size, frequently associated with a bulging of the cornea, and in some cases by a herniation of the growth through the outer surface of the eye (Figs. 6 and 9). In Fig. 9 it may be seen that the tumor tissue has invaded between the corneal layers with the result that it is almost completely surrounded by stratified squamous epithelium. Fig. 10 gives a higher magnification of this transplant and shows the "fingers" of epithelial cells invading the tumor. After about 15 days' residence in the rodent eye the size of the tumor decreases and eventually no trace of the growth remains. A corneal scar may persist, however. Successful transfer of growths from mice to chicks has not been achieved after the 15th day. Serial passage of the Rous sarcoma in the mouse has not been possible as yet beyond the second rodent transfer.

Histologically the tumor in the mouse possessed no features distinguishing it from that seen in stock Rous tumor material (Figs. 5, 7, and 8). Fig. 11 represents the architecture of the tumors of the mouse material in chicks. It may be seen that there is little change from the original growths. Some of these sarcomas contain considerable mucin.

Properties of the mouse passage tumor in chicks.—Of the 50 chicks that received transplants from the mouse eye, 43 developed tumors; 31 of these were localized in the region into which the eye transplant was placed. Eight of the 43 individuals possessed tumors also in the lungs, and 3 others showed extension of the local growth to the liver. Nine birds receiving the sarcoma directly from the rodent possessed hemorrhagic lesions of varying intensities, whereas 2 chicks developed periosteal growths following inoculation of the anterior chamber transplants. There was no correlation between the length of time the sarcoma remained in the rodent and the type of lesions present in the chick.

Probably the most significant point to be noted in the behavior of the mouse passage strain in chicks is that the incidence of bone lesions among the birds inoculated with filtrates is low (Table III). Fig. 12 illustrates the histology of these lesions and it may be seen that there is no essential difference between this and the periosteal growths of the guinea pig passage tumor (6). On one occasion, however, there was evidence of a marked increase in cortex bone associated with the periosteal tumor (Fig. 13). Fig. 14 shows a higher magnification of this tissue and it can be seen that some of the osteoid tissue is undergoing degeneration.

The incidence of hemorrhagic disease in these

TABLE II: THE PROBABILITIES THAT WEIGHTED MEAN AGES OF THE BIRDS BEING COMPARED DO NOT VARY BEYOND THE LIMITS OF RANDOM SAMPLING

Chicks inoculated with tumor, Strain	Inoculum	Birds with lesions	No. of birds inoculated	Weighted mean age	Standard error	Probability*
Stock Rous	Filtrates	Bone	23	14.87 ±	2.05	< 0.01
Guinea pig			52	8.73 ±	0.69	< 0.01
Mouse			41	9.15 ±	0.68	> 0.60
Stock Rous	Filtrates	Hemorrhagic	78	11.83 ±	0.94	< 0.01
Guinea pig			97	7.76 ±	0.41	< 0.01
Mouse			148	7.41 ±	0.40	> 0.50
Stock Rous	Cell suspension	Hemorrhagic	111	16.57 ±	1.21	< 0.01
Guinea pig			84	11.93 ±	0.68	< 0.01
Mouse			33	5.09 ±	0.60	< 0.01
Stock Rous	Filtrates	All manifestations of virus infection	105	11.90 ±	0.84	< 0.01
Guinea pig			121	7.65 ±	0.39	< 0.01
Mouse			237	7.34 ±	0.31	> 0.50
Stock Rous	Cell suspension	All manifestations of virus infection	445	15.37 ±	0.52	< 0.01
Guinea pig			153	11.22 ±	0.66	< 0.01
Mouse			179	6.03 ±	0.28	< 0.01

*The probabilities were determined by means of the "t" test.

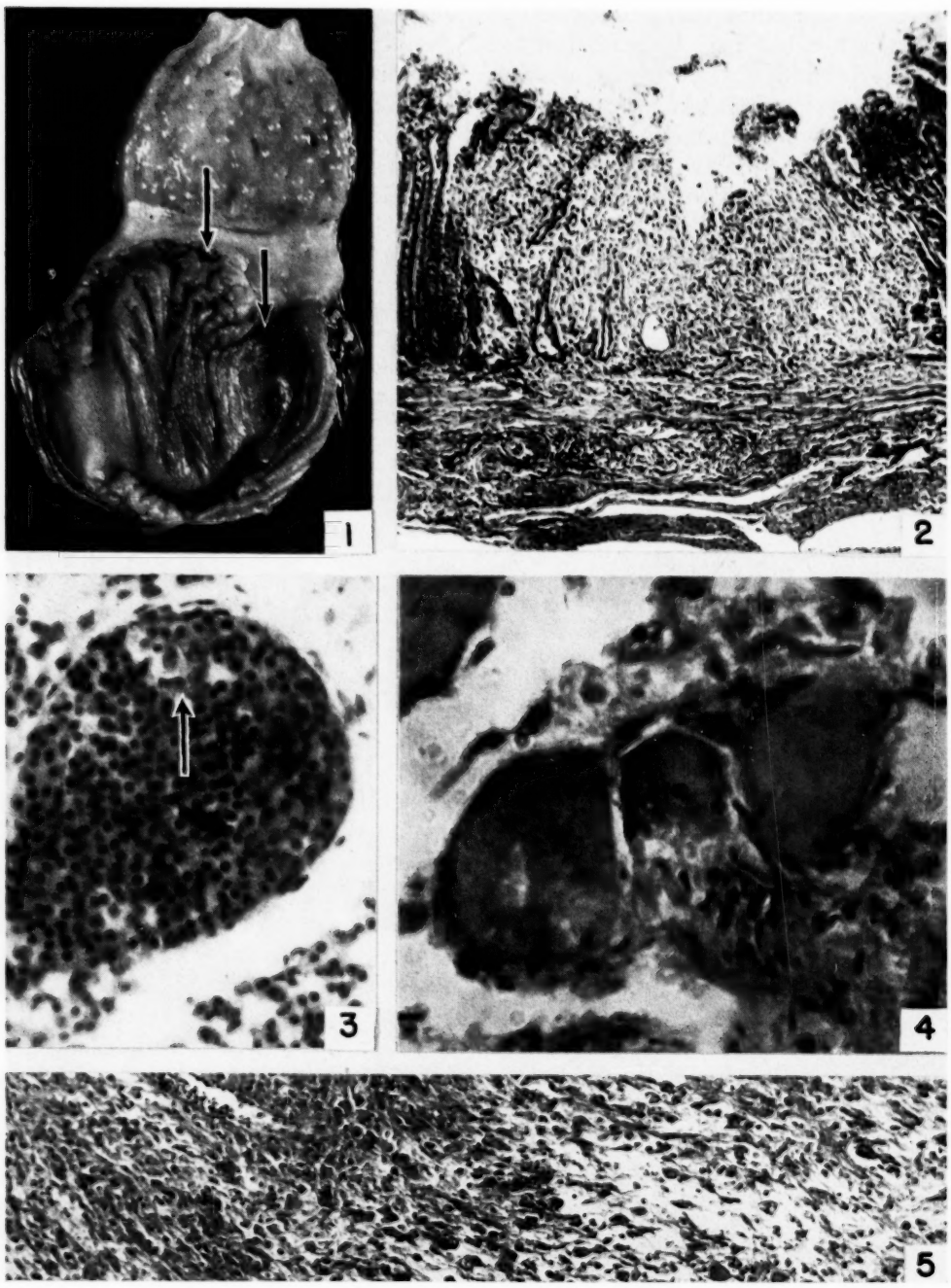

FIG. 1.—Ulceration of gizzard mucosa due to infiltration of cells of Rous sarcoma of guinea pig passage strain.

FIG. 2.—Invasion and replacement of cells of gizzard mucosa by sarcoma cells. Mag. × 100.

FIG. 3.—Embolus of sarcoma cells in pulmonary vessel of chick inoculated with guinea pig passage material. Mag. × 800.

FIG. 4.—Invasion of muscle cell by malignant fibroblasts of guinea pig passage tumor. Mag. × 800.

FIG. 5.—Histology of Rous chicken sarcoma used as control material. Mag. × 160.

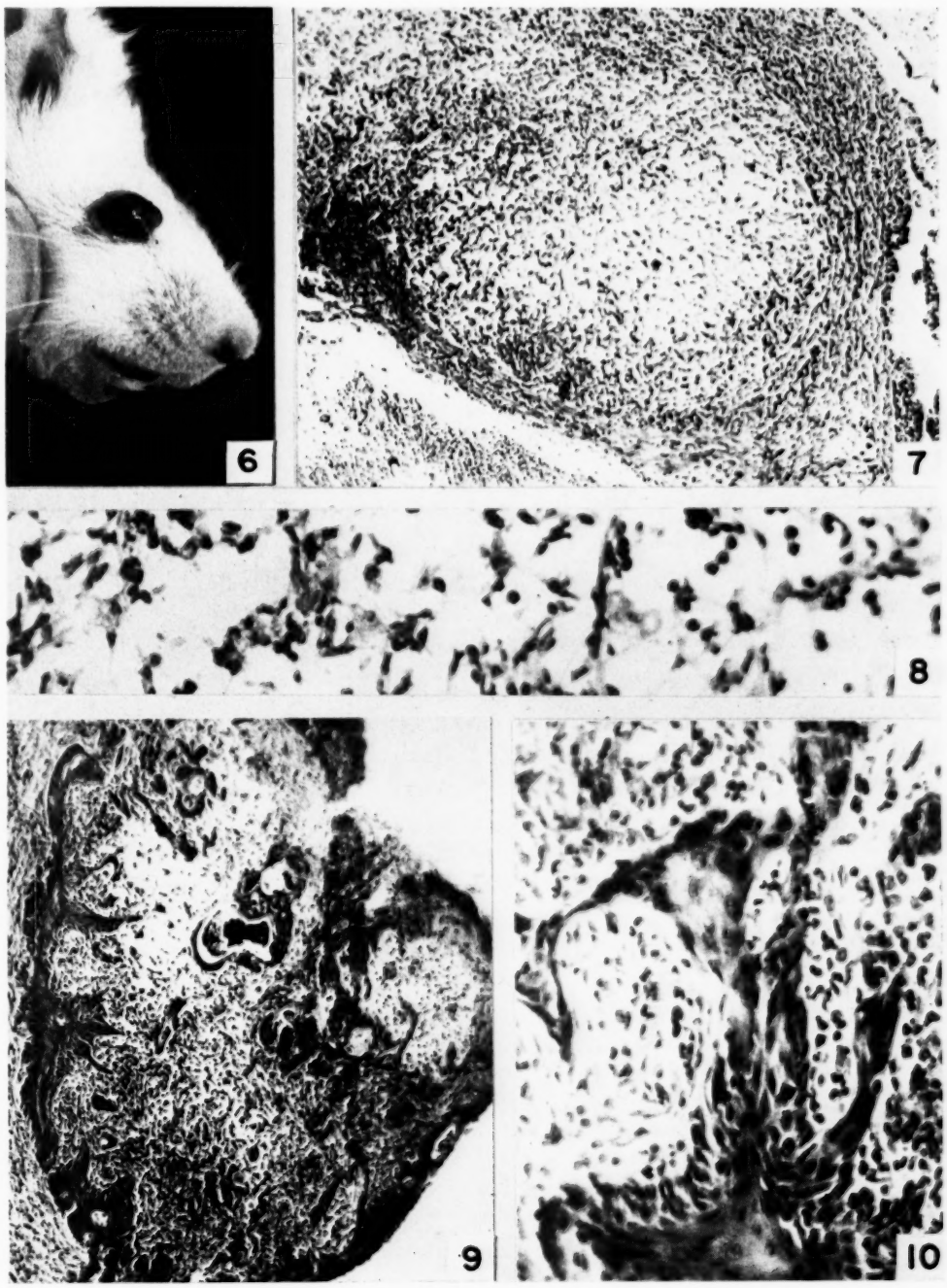

FIG. 6.—The Rous sarcoma growing in anterior chamber of mouse eye.

FIG. 7.—Histological architecture of Rous sarcoma growing in anterior chamber of the mouse eye. Mag. × 85.

FIG. 8.—Tissue seen in Fig. 7. Mag. × 600.

FIG. 9.—Herniation of sarcoma in mouse eye through cornea. The tumor has invaded between layers of cornea and has ulcerated through outside surface. Note "fingers" of stratified squamous epithelium entering the tumor. Mag. × 85.

FIG. 10.—Section seen in Fig. 9. Mag. × 365.

birds inoculated with mouse passage tumor may be seen in Table V, while the distribution of the intensities of the hemorrhagic lesions is presented in Table VI. Those individuals inoculated with cell suspensions of this tumor strain possessed growths in the majority of instances at the injection site only (Table VII). This was likewise true, but not to the same degree, for those receiving filtrates of the sarcoma. On the other hand 6.7 per cent of the birds with filtrates gave no tumors at all.

arose as to whether there was a greater likelihood of multiple bone lesions occurring in birds inoculated with the guinea pig strain than in those injected with either the mouse or control tumors. Table IV shows that no matter what the origin of the inoculum may be, the bird developing one bone lesion has the same chance, within the limits of random sampling, of developing more.

Of 445 chicks inoculated with cell suspensions of the stock Rous growth only 7 developed bone tumors. Two birds in the mouse passage group

TABLE III: A COMPARISON OF THE INCIDENCES OF BONE TUMORS IN CHICKS INOCULATED RESPECTIVELY WITH FILTRATES FROM THE THREE TUMOR VIRUS STRAINS

Filtrate from*	Birds with bone lesions		Birds without bone lesions		Total no. of birds inoculated	Probability†
	No.	%	No.	%		
Guinea pig passage tumor	52	43.0	69	57.0	121	<0.01
Mouse passage tumor	41	17.3	196	82.7	237	<0.01
Stock Rous tumor	23	21.9	82	78.1	105	0.33

*See text for explanation of terms.
†The probability that the two groups being contrasted are random samples from the same population.

A comparison between the properties of the 3 strains of the Rous sarcoma virus.—Since the types of clinico-pathological manifestations of the Rous virus infection may depend upon the age of the host, the weighted mean age of the birds in each of the groups to be compared appears in Table II. It can be seen that the ages of the birds injected with the stock Rous strain were significantly different from those inoculated with the other two. However in only two instances did the mean ages of the chicks inoculated with the guinea pig and mouse strains vary beyond the limits of random sampling and these received cell suspensions.

A study of the effects of the two mammalian environments upon the properties of the Rous tumor virus was achieved by a detailed comparison of the frequencies of the lesions described above. Table III shows that significantly more bone lesions occurred in chicks inoculated with filtrates of the guinea pig strain of tumor than in chicks injected with either of the other two virus strains. On the other hand the mouse passage material did not differ significantly from the stock Rous control in this regard. The incidence of 21.9 per cent periosteal tumors in the stock Rous-infected birds suggests that the virus may undergo spontaneous alteration in its tissue specificities.

Occasionally all 3 of the virus strains produced more than one bone lesion in chicks injected. In fact, in 1 bird as many as 7 bone tumors were present. The locations included not only the long bones but also the pelvis and skull. The question

and 6 in the guinea pig passage series possessed similar lesions, 179 and 153 individuals being included in the respective groups.

Another property of the 3 virus strains which lends itself to comparison is the relative ability of the infectious agents to produce hemorrhagic disease in their respective hosts. Table V represents the frequencies, with their corresponding percentages, of hemorrhagic lesions which appeared in individuals of the three bird populations. It can be seen that in general there is a greater tendency for the guinea pig passage virus to produce hemorrhagic lesions than either the mouse or stock agents. When filtrates of the growths were used, the number of birds which showed these lesions was slightly less in the group receiving mouse material than in the control population. Definitely more chicks showed hemorrhages following injection of guinea pig passage tumor than did those getting mouse material. There was essentially no difference between the guinea pig and control viruses when

TABLE IV: THE INCIDENCE OF MULTIPLE BONE LESIONS OCCURRING IN CHICKS INOCULATED WITH FILTRATES OF THE VARIOUS STRAINS OF THE ROUS SARCOMA

Filtrate from	No. of birds with 1 bone lesion	No. of birds with more than 1 bone lesion	Total No. of birds with bone lesions	Probability
Guinea pig passage tumor	32	20	52	0.80
Mouse passage tumor	19	22	41	0.25
Stock Rous tumor	11	12	23	0.68

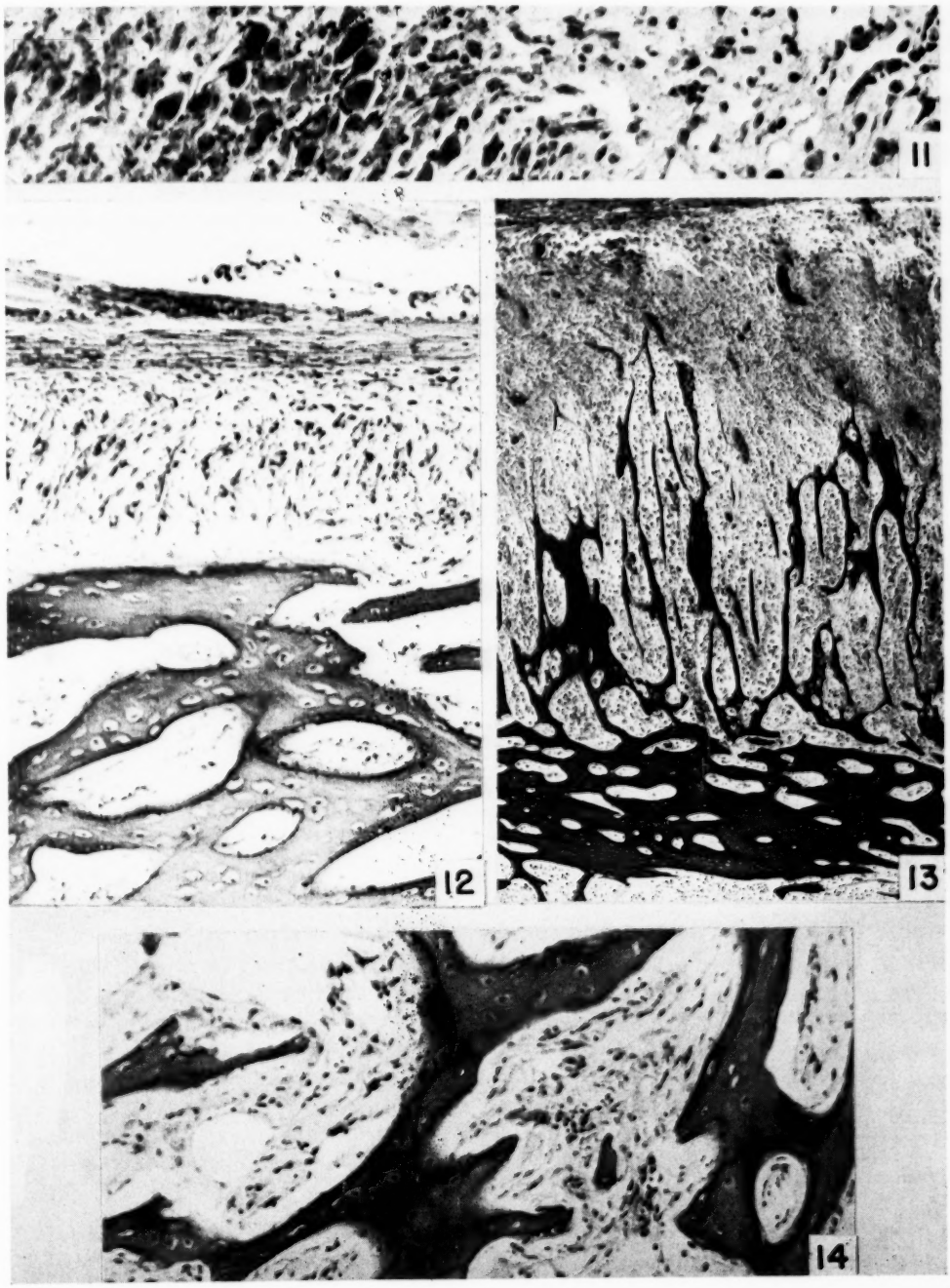

FIG. 11.—Sarcoma in chick inoculated with transplant from mouse eye following residence in rodent of 15 days. Mag. × 240.

FIG. 12.—Periosteal tumor in chick inoculated with mouse passage tumor virus. Histologically the periosteal growths were similar regardless from which of the three strains the virus was taken. Mag. × 250.

FIG. 13.—Bone tumor in which new cortex has been laid down. This is now undergoing regression. The bird was injected with mouse passage tumor virus. Mag. × 85.

FIG. 14.—Tumor seen in Fig. 13. Mag. × 250.

filtrates were used. With cell suspensions, however, the guinea pig passage virus produced definitely more hemorrhagic lesions than either of the other two strains.

What the effect of age of the birds injected may have on these results is not clear, but at most it is probably a minor factor in the range we are working. This is borne out by 3 points: one, the fact that birds injected with stock and mouse strains varied in age but not in the frequency of bone and hemorrhagic lesions; two, birds with and without hemorrhagic lesions within a specific inoculated group were essentially the same age; and three, the variations between the frequencies of the lesions obtained were significant even though the birds receiving guinea pig and mouse strain viruses did not differ significantly in age.

Attempts have been made to quantitate the intensity of these lesions. The extent of hemorrhagic disease has been arbitrarily graded according to three classes: 1, 2, and 3. Class 1 includes those birds showing a few small hemorrhagic blebs scattered over one organ, or 1 large bleb on a single organ. Class 2 represents individuals possessing scattered small lesions over more than 1 organ or several large blebs only on 1 organ. Finally those chicks in class 3 show large lesions in abundance distributed over many organs. From Table VI it can be seen that the frequencies among the chicks inoculated with filtrates of the respective tumor strains show little or no difference in classes 1, 2, and 3 for the three groups. However, in those chicks injected with the cell suspensions, the guinea pig passage material has a greater tendency to produce the most severe manifestations, in contrast to the other two strains.

TABLE V: A COMPARISON OF THE INCIDENCE OF HEMORRHAGIC LESIONS IN CHICKS INOCULATED WITH VARIOUS STRAINS OF THE ROUS SARCOMA

Filtrate from	Birds with hemorrhagic lesions		Birds without hemorrhagic lesions		Total no. of birds inoculated	Probability
	No.	%	No.	%		
Guinea pig passage tumor	97	80.0	24	20.0	121	<0.01
Mouse passage tumor	148	62.5	89	37.5	237	0.41
Stock Rous tumor	78	74.3	27	25.7	105	0.04
Cell suspension from						
Guinea pig passage tumor	84	55.0	69	45.0	153	<0.01
Mouse passage tumor	33	18.4	146	81.6	179	<0.01
Stock Rous tumor	111	24.9	334	75.1	445	0.13

A comparison between virus strains of the various locations of tumors developing after either intravenous plus intramuscular inoculation of filtrates on the one hand, or intramuscular injection of cell suspensions on the other, may be seen in Table VII. The incidence of individuals showing only local lesions at the site of inoculation did not vary significantly among the three groups of birds when filtrates were administered. However, in the case of cell suspensions, each group differed from one another beyond the limits of random sampling, the guinea pig strain eliciting less local tumors than the stock virus, and the mouse strain showing the greatest tendency for such localiza-

TABLE VI: THE DISTRIBUTION IN PER CENT OF THE INTENSITY OF HEMORRHAGIC LESIONS AMONG CHICKS INOCULATED WITH VARIOUS STRAINS OF THE ROUS SARCOMA

Filtrate from	Intensity of hemorrhagic lesions*			Total birds inoculated	Probability
	1	2	3		
Guinea pig passage tumor	10.3	24.7	65.0	97	0.04
Mouse passage tumor	24.4	18.9	56.7	148	0.02
Stock Rous tumor	25.6	25.6	48.8	78	0.46
Cell suspension from					
Guinea pig passage tumor	19.0	19.0	62.0	84	<0.01
Mouse passage tumor	45.6	27.2	27.2	33	<0.01
Stock Rous tumor	43.2	28.8	28.0	111	0.76

*See text for explanation.

tion. Those birds possessing, in addition to local tumors, growths in the lungs and those birds with extension of their local sarcomas to the liver were not compared statistically since it was felt that these manifestations of infection were too dependent upon the technic of injection to represent true properties of the infectious agents. In spite of the incidence of 11.4 per cent of chicks showing the general spread of tumors throughout the body in the mouse passage strain, this value did not vary significantly from the frequencies of similar lesions with the control tumor virus. The frequency of these lesions in the birds inoculated with cell suspensions was considerably less in all the three bird groups than when filtrates of the tumors were used.

Since bone lesions indistinguishable histologically from those seen in the chicks injected with rodent passage tumors were present in the control birds, it would suggest that some alteration in tissue specificities of the virus occurs naturally and the guinea pig passage merely speeds up this process while mouse passage does not alter it at all.

TABLE VII: THE FREQUENCY OF VARIOUS TYPES OF TUMOR RESPONSES IN BIRDS INOCULATED WITH CELL SUSPENSIONS OR FILTRATES OF VARIOUS ROUS TUMOR STRAINS

	Percentage of birds in the 3 groups with tumors					
	Stock Rous tumor		Guinea pig tumor		Mouse tumor	
Birds with*	Cell susp.	Filt.	Cell susp.	Filt.	Cell susp.	Filt.
Local tumors	72.4	62.8	48.4	62.8	87.8	74.7
Local tumors plus lung growths	15.0	27.6	32.0	8.4	7.3	4.2
Local tumors plus liver growths	11.9	0.9	17.6	8.4	3.3	3.0
Generalized tumors	0.7	6.8	2.0	5.7	1.1	11.4
No tumors at all†	0.0	1.9	0.0	14.7	0.5	6.7
Total number of birds observed	445	105	153	121	179	237

*See text for explanation.
†Those birds not showing tumors had other manifestations of Rous virus infection.

in the other two populations. One significant observation made, recorded in Table VII, is that the Rous sarcoma virus apparently lost some of its ability to produce tumors in chicks by virtue of its sojourn in the guinea pig. Approximately 14 per cent of the individuals receiving the guinea pig passage agent failed to develop growths although other manifestations of the virus infection were present. The percentage of birds injected with the mouse passage filtrate not developing tumors, represented a frequency that was not significantly different from that observed in the control chicks.

DISCUSSION

It has been possible to grow the Rous chicken sarcoma in the anterior chamber of the guinea pig eye as well as in the eye of the mouse. The subsequent inoculation of the filtrates and cell suspensions of these two strains of sarcoma into chicks has yielded information suggesting that the causative virus that has passed through the guinea pig has become altered in its tissue specificities, as seen by the increase in the incidence of periosteal lesions. On the other hand, the agent that had sojourned in the mouse eye did not show an incidence of bone lesions in avian hosts differing significantly from that found in the birds injected Regardless of the tumor strain from which the filtrates are obtained, the probability of inoculated chicks developing more than one bone lesion is not significantly different in the three host groups. This further suggests that the alteration in the guinea pig virus may be just an accentuation of a natural phenomenon.

Guinea pig passage tumor virus had a greater tendency to produce hemorrhagic disease in chicks than had either of the other two virus strains. This was particularly clear when birds were inoculated with cell suspensions. Moreover these individuals of the guinea pig passage strain possessed more severe hemorrhagic lesions than the members of the other two groups.

Although the incidence of solitary lesions at the site of inoculation among the birds of the three populations was not significantly different when filtrates were used, there was a definite tendency with cell suspensions for the guinea pig virus to produce less localization than the mouse passage agent. In fact there was a suggestion of a greater spread of lesions in the control infections than in the birds injected with mouse passage strain. Although the three strains of viruses differed regarding the incidence of tumors at the site of inoculation, there was no greater tendency for any one strain to give generalized tumors throughout the

body. The guinea pig as well as the stock Rous virus may have a greater tendency to produce tumors in the lungs, and growths in the liver in addition to their local lesions than the mouse strain virus, albeit these data were not analyzed statistically for reasons stated above. Finally, definitely fewer birds developed tumors on inoculation with the filtrate of the guinea pig passage virus, yet these chicks possessed other manifestations of the Rous infection. Study of this non-tumor group showed that age was not a factor. It appears, therefore, that by virtue of the guinea pig passage the Rous virus has lost some of its ability to produce tumors in chicks when filtrates are used. This might suggest an alteration in the species specificities of the Rous virus.

Since Duran-Reynals (1) has shown that hemorrhagic lesions in the chick are the result of an interplay of virus virulence and host resistance and that this host resistance may be closely associated with age, the present results suggest that the potency of the Rous virus has been increased by guinea pig passage. Whether this alteration in potency is a qualitative or quantitative change can not be said at present. Furthermore, the Rous agent was so altered in its tissue specificities by its sojourn in the guinea pig that it produced bone tumors in greater abundance. On the other hand, the mouse passage virus possessed properties, concerning potentialties for the production of hemorrhagic and bone lesions, which were more closely allied with those observed for the stock Rous agent. On working with the mouse virus strain one gains the impression that actually the potency of this virus is less than that of the control. The only statistical evidence that might support this theory is the observation that cell suspensions of the mouse virus produce more solitary tumors in birds at the site of injection than either of the other two strains.

The age of the host, within the range of 1 to 60 days, is probably not an important factor in the manifestation of the various lesions produced by the virus strains. While the mean ages of the birds in the various groups vary significantly from each other, those showing wide discrepancy of age gave no significant differences in the incidence of bone and hemorrhagic lesions. On the other hand, the birds receiving guinea pig and mouse strain virus showed significantly different frequencies of these lesions, yet these chicks did not vary in age beyond the limits of random sampling.

The behavior of the Rous sarcoma in the mouse eye is practically identical with that of the sarcoma in the anterior chamber of the guinea pig eye, with the possible exception that its period of growth is longer. Mammalian cancer in these environments has been described by Greene (4 and 5), and the activities of these growths in contrast with those of the Rous sarcoma have been discussed (6). With reference to the behavior of human tumors in the mouse eye Greene (5) has noted that great difficulty is encountered in transplanting these growths directly from man to mouse. However, following growth in the guinea pig eye the human neoplasm will grow readily in the mouse, eventually killing the animal.

SUMMARY AND CONCLUSIONS

By the inoculation of the Rous Chicken sarcoma into the anterior chamber of the eyes of mice and guinea pigs, studies have been made possible concerning the effects of these two rodent hosts upon the specificities and potencies of the respective tumor viruses. It was found that the guinea pig had a more pronounced influence upon these properties than the mouse. In the former, changes were manifested in chicks by the increase of the incidence of periosteal and hemorrhagic lesions, and by the decrease in the appearance of localized growths at the inoculation site of the filtrates. On the other hand the virus that was passed through mice was essentially similar in properties to the agent experiencing no rodent passage.

These results are interpreted as suggesting that residence of the Rous tumor in the guinea pig eye increased the potency of the virus and altered its tissue and species specificities. By contrast the passage of the sarcoma through mice had no such effect. In fact there was a possibility that the potency of the virus was actually decreased by this procedure.

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Organophilic Tendencies of Two Transplantable Tumors of the Mouse*†

Arthur M. Cloudman, Ph. D.

(*From the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine*)

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INTRODUCTION

Relationships between the transplanted tumor and its host.—A growing neoplasm that will eventually cause the death of the host may or may not develop daughter colonies, or metastases. When a mouse tumor is successfully transplanted into new hosts the same tendency either to metastasize in a certain percentage of the host mice or to remain localized is apparently shown by the tumors of later tumor tissue transplant generations. In other words some implanted tumors characteristically remain at the site of inoculation while others may not only develop at the foci of transplantation but also be transferred to other parts of the body. Certain human neoplastic growths have characteristic sites for metastases such as the bones in some cases of carcinomas of the breast and of the prostate. The tumors of mice in general may metastasize by means of tumor cell emboli that most commonly go to the lungs. Ewing states "That tissue—and tumor—cells pass through the pulmonary capillaries has frequently been demonstrated, and in certain tumors, especially the lymphosarcomas, general metastases develop in this way. Passing the lungs the embolic cells tend to lodge in organs with feeble circulation, such as liver, bone marrow, and subcutaneous tissue." (3)

This explanation is not sufficient to account for the relationships between the different host types and the transplantable tumor that will be analyzed in this paper. These data present a comparative study of two different tumors. However, the primary objective here is to analyze the host-tumor relationship where the same transplantable tumor has been observed in a variety of different genetically controlled host types. The systemic response for each host type has been studied in order to determine which internal organs show gross evidence of tumor involvement concurrent with the growth of neoplasms at the subcutaneous implantation site. It is hoped that these data will clarify the following points: (a) If a tumor metastasizes, does it involve the same organs in all types of hosts? (b) Does the percentage of metastastic growths of the same tumor vary for different types of hosts? (c) Does this frequency of metastases vary for the same organs within a host type? (d) If an organophilic tendency is associated with tumor metastases, is this dependent upon the tumor, the host, or upon a host-tumor relationship?

Spontaneous neoplasia in C57 leaden strain mice.—The C57 leaden strain mice arose by mutation from the C57 brown strain (7). The total tumor incidence has not been tabulated, but there are certain general observations concerning spontaneous tumors that can be stated here. Although several hundred careful autopsies were carried out on old leaden mice, only 1 tumor of mammary gland origin has been seen during a period of 12 years. This mammary tumor was nontransplantable and on sectioning proved to be an adenoma. Primary lung tumors have been nearly as rare as tumors of the mammary gland. We have found fibrosarcomas and leukemias somewhat more frequently, probably at about the same rate as has been recorded for the C57 black stock (6). If any one body site in the leaden mice is the most frequently involved by spontaneous tumors, it would appear to be the liver. The growths found in this organ have usually been either parenchyma cell tumors or reticuloendotheliomas (histiocytomas). A representative of each of these two tumor types from the liver was selected for the following studies by transplantation.

MATERIALS AND METHODS

Types of animals used.—Several types of mice were employed. These were strains C57 black line 6 (B), C57 brown lines *a* and *cd* (Br^a and Br^{cd}), C57 leaden (L), dilute brown (dba), and albino (A). First generation hybrids between several of these strains were also tested. The C57 strains are not closely related; their origin from a common hybrid ancestry is diagrammatically shown.

Tumors employed.—A liver carcinoma (C954) and a reticuloendothelioma (C198) were studied

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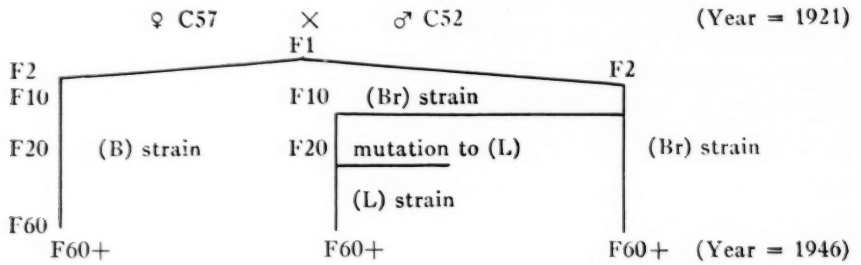
with respect to their organophilic tendencies following transplantation. Tumor C954 was first seen in 1940 as a spontaneous tumor of the parenchyma cells of the liver. It arose in an old male of the leaden strain. This carcinoma has been carried in leaden mice for more than 60 tumor transplant generations. The other spontaneous tumor, C198, also originally involved the liver of a leaden strain mouse. It was observed in an old breeding female that was autopsied in 1936 (1).



In 10 years this tumor passed through 145 tissue transplant generations in leaden strain host mice.

Method of tumor transfer.—The usual trocar method was employed and the implants were routinely made into the subcutaneous tissues in the right axillary region. The neoplastic liver tissue was the source of the tumor tissue used in the initial transfer of both spontaneous tumors C954 and C198 to other leaden strain mice. After the first transfer the source of the tissue for subsequent inoculations differed for these 2 tumors.

For more than 10 years the reticuloendothelioma C198 had been carried in the leaden strain from host to host by injecting fragments from the tumorous livers rather than from the subcutaneous tumors. This practice was necessary at first because only 10 mice of the 111 inoculated in the first 5 transfer generation developed any subcutaneous masses at the site of injection in the right axillary region. These neoplasms were but a few millimeters in diameter. Nevertheless, all the leaden strain mice injected subcutaneously, developed tumor lesions in the liver. These lesions were grossly and microscopically identical with the spontaneous reticuloendothelioma. The tumorous livers proved to be a dependable and logical source for the tumor tissue needed for additional inoculations.

The carcinoma of liver parenchyma cells (C954) was originally injected subcutaneously into 12 young leaden strain mice. Six months later all the mice but 1 were discarded as negative. This mouse responded a month later by developing a nodule at the implantation site, which resembled the primary tumor and was used as the source of tumor tissue for further inoculations. After the first transfer, tumor C954 has grown subcutaneously in approximately 100 per cent of all leaden strain mice inoculated.

Method of recording data.—Complete necropsies were performed upon practically all these mice when they became moribund from advanced tumor growth. If the animals were resistant to tumor inoculations they were autopsied at the end of 6 to 9 months. Mice developing subcutaneous tumors at the implantation site have been compared to determine the organophilic tendencies of the same tumor in different host backgrounds. Detailed observations were kept on the thoracic and abdominal structures that gave any evidence of containing foci of tumor metastases. These records always included data on the involvement of lungs, liver, kidneys, gonads, spleen and lymph nodes.

When question arose as to whether tumor C198 was present, the tissue was either sectioned and stained or tested by inoculating it into a group of susceptible leaden hosts. The former method was useful in cases of early tumor involvement in young adult mice, and both methods were used when the hosts lived long enough for new tumors to arise spontaneously. If the prepared sections were histologically identical with C198 and/or the transplanted fresh tumor tissue developed masses indistinguishable from this tumor in leaden strain hosts, the case in question was called a positive growth of C198. In this way both morphological and physiological tests were carried out.

RESULTS

A. Local Response to Injections of Tumor Tissue

Transplantation of C954 and C198 into (L) strain hosts.—The general reactions of the susceptible leaden host mice to subcutaneous implants of these 2 tumors are shown in Table I. A sample of 450 mice showed that liver cell carcinoma C954 always grew progressively but remained localized at the subcutaneous site where the tumor was in-

jected. No cases of extension of this tumor to another part of the body occurred. On the other hand, the reticuloendothelioma C198 showed both internal and external manifestations of tumor growth in 100 per cent of the leaden strain mice taken as a representative sample, following subcutaneous implantations of C198, of the host-tumor relationship. The particular organs that responded to subcutaneous implants of tumor C198 are taken up later in this paper. Suffice it to note here, that only the reticuloendothelioma C198 showed a characteristic tendency to extension beyond the site of inoculation. For this reason carcinoma C954 was not analyzed further. However, tumor C198 was studied more intensively for the presence of organophilic tendencies.

TABLE I: GENERAL RESPONSE OF LEADEN (L) STRAIN MICE TO SUBCUTANEOUS IMPLANTS OF TWO SPONTANEOUS TUMORS THAT AROSE IN ANIMALS OF THE (L) STRAIN

Tumor inoc.	Type of tumor inoculated	No. (L) mice inoc.	Subcut. response, %+	Internal response, %+
C198	Reticulo-endothelioma	1,179	100	100
C954	Carcinoma of liver	450	100	0

Inoculation of C198 into different pure strains of mice.—Animals from several inbred strains of mice were inoculated with this tumor (Table II). No mice from pure stocks, other than leaden, developed C198 following inoculation under the usual transplant conditions. These data were spread over a considerable time interval and included different tumor transplant generations.

TABLE II: LOCAL RESPONSE OF DIFFERENT TYPES OF MICE TO SUBCUTANEOUS INJECTIONS OF TUMOR C198

Strain	No. of mice	% + Subcutaneously Observed	% + Subcutaneously Expected
L	1,179	100	100
B	130	0	0
B of (L) − (B)	34	91	0
B with C198a	98	84	0
Br^{a} and Br^{cd}	68	0	0
dba	16	0	0
A	56	0	0
F_1 (L × B)	85	100	100
F_1 (L × Br^{cd})	42	100	100
F_1 (B × Br^{cd})	122	100	0
F_1 (B × Br^{a})	60	100	0
F_1 (B × dba)	23	91	0
F_1 (B × A)	36	81	0

(L) − (B) = Mice in parabiotic union.
B with C198a = B mice inoculated with C198 that had grown successfully in B host while in parabiotic union.

Implantation of C198 into parabiotic B hosts.—This tumor has been grown in some members of the C57 black stock under exceptional conditions. Black strain mice surgically joined in parabiotic union with the susceptible leaden mice became susceptive to C198 implants. About 90 per cent of B strain animals tested by this method grew this leaden strain tumor. An earlier publication presented this work in more detail (2).

Effect of parabiotically altered host upon C198 implant.—Once a C57 black mouse had progressively grown C198, while the mouse was under some influence activated by parabiosis, the tumor itself often showed physiological evidence of a change. This became evident when fragments of subcutaneous masses of C198 tumor were taken from positive parabiotic C57 black mice and injected into the normal untreated C57 black strain animals. Many black strain mice responded to this altered tumor, which will be called C198a. Table II shows that 84 per cent of such mice were susceptive to inoculations of C198a. No histological difference has been observed between C198 and C198a.

Inoculation of C198 into hybrid hosts.—As was expected, all first generation hybrid mice between the susceptible leaden strain and other pure strains of mice grew implants of tumor C198 from leaden donors. The data on two such crosses can be seen in Table II. All of these hosts had subcutaneous masses.

Both parents of hybrids from B mice and strains other than L were resistant to C198. Based on the genetic theory of transplantation, therefore, the expected response to injections of this tumor was very different from the data (5) observed. Nearly all these hybrids were susceptible to the tumor when it was injected subcutaneously.

In 4 crosses between C57 black mice and other resistant strains, 80 to 100 per cent of the first generation hybrids were positive to inoculations of the original C198 tumor (Table II). Leaden hosts were used as donors for all the C198 tumor material inoculated into these hybrids.

TABLE III: METASTASES OF SUBCUTANEOUS IMPLANTS OF C198 TO INTERNAL ORGANS OF DIFFERENT HOST-TYPES

Strain	Autopsied with + subcut.	Percentage with internal metastases
L	71	100
B of (L) − (B)	26	62
B with C198a	55	2
F_1 (L × B)	45	100
F_1 (L × Br^{cd})	42	100
F_1 (B × Br^{cd})	114	100
F_1 (B × Br^{a})	46	100
F_1 (B × dba)	17	65
F_1 (B × A)	21	59

B. SYSTEMIC RESPONSE TO SUBCUTANEOUS TRANSPLANTS OF C198

1. *General systemic response.*—Data from the pure and hybrid mice autopsied after growing C198 subcutaneous implants are shown in Table III. The percentages of host mice that showed

metastases of this tumor to some internal organs were tabulated. The findings for specific organs in different host types are given in detail in Tables IV and V.

2. *Specific systemic responses. Metastases of C198 in the L and B strains.*—Table IV shows the internal sites that were grossly involved by tumor C198 after this neoplasm had been successfully implanted into the right axillary subcutaneous tissues. In the leaden hosts the liver always developed tumorous foci. Also 76 per cent of the lungs showed typical, hemorrhagic areas containing C198 tumor cells and 65 per cent of the spleens had one or more white tumor nodules.

When the C57 black strain hosts were in parabiotic combination with leaden strain mice autopsies of the C57 black strain mice with subcutaneous masses showed that 58 per cent had liver nodules; 23 per cent, lung involvement; and 15 per cent, tumors in the spleen. However, mice of this same black strain showed but very slight evidence of organophilic tendencies when they were not in parabiotic union but received and grew implants of the altered tumor, C198a. As stated previously, this tumor became changed during growth in a C57 black parabiotic host.

Metastases of C198 in F_1 hybrids.—First generation hybrid mice, where one parent was from the leaden stock, developed 100 per cent tumor metastases in their livers following subcutaneous inoculations with C198. These findings on metastases were identical with those for the leaden stock. Many of these hybrids showed pulmonary foci of this tumor. In addition to this nearly 100 per cent of the F_1 (L × Br^{cd}) of both sexes had multiple metastatic tumor nodules in their kidneys and all the females had ovarian metastases of C198. The ovaries were not multinodular but consisted of one rounded mass frequently more than 1 cm. in diameter. The male gonads were not grossly involved by C198. Here the spleen did not show a single metastatic tumor nodule. However, in the F_1 (L × B) hybrids with the same treatment about half the mice exhibited extensive growths of C198 in their spleens. Only a few of these hybrids had ovarian and nephric metastatic nodules of the tumor C198.

The C57 black strain mice were crossed with several pure stocks besides the leaden mice and most of these individuals developed masses at the site of tumor implantation (Table II). The organophilic tendencies observed in mice with these implants are shown in Table IV. When B mice were crossed to Br^{cd} mice the findings in the internal organs of the F_1 hybrids, following the subcutaneous implants of C198, were very much like those observed in the F_1 (L × Br^{cd}) mice except that fewer animals showed liver metastases. Nearly all these and the F_1 (B × Br^a) hybrids developed metastases in the form of large tumorous ovaries and multiple white tumor nodules in their kidneys. On the other hand, F_1 (B × dba) and F_1 (B × A) hybrids had no cases of tumor invasion of the ovaries and a relatively small percentage of these hybrids has C198 nodules in their kidneys. (Table IV).

TABLE IV: ORGANOPHILIC TENDENCIES OF METASTASES OF C198 IN VARIOUS HOST TYPES BEARING SUBCUTANEOUS IMPLANTS OF TUMOR

Strain	Autopsied with subcut.	Percentage with internal metastases	Liver % +	Lungs % +	Kidneys % +	Spleen % +	Ovaries % +	Lymph nodes % +
L	71	100	100	76	0	65	0	6
B of (L)−(B)	26	62	58	23	0	15	0	12
B with C198a	55	2	2	0	0	0	0	0
F_1 (L × B)	45	100	100	91	7	51	17	20
F_1 (L × Br^{cd})	42	100	100	48	98	0	100	24
F_1 (B × Br^{cd})	114	100	77	49	91	11	89	43
F_1 (B × Br^a)	46	100	50	76	98	15	96	63
F_1 (B × dba)	17	65	18	12	18	29	0	41
F_1 (B × A)	21	59	30	11	8	15	0	19

Hepatic response to C198 metastases.—The livers of mice with foci of C198 did not present the same gross characteristics in all host animals. The response of this organ is further analyzed in Table V. Metastatic tumors of C198 sometimes formed discrete white nodules (Nod.) when present in the liver, or the organ might be swollen and hemorrhagic (S. H.) with a diffuse growth of tumor cells but no distinct nodules. Sometimes a liver showed a swollen, hemorrhagic condition with tumor nodules. However, each type of host usually had liver metastases that were fairly characteristic for that group of animals. Table V shows that all the leaden mice had liver metastases. Nearly all these livers were swollen, hemorrhagic and had diffuse growths of metastatic tumor cells. Approximately one-third had distinct, multiple, small tumor nodules. About half the C57 black mice that were in parabiotic union with leadens, or B of (L) − (B), and that grew subcutaneous

masses produced C198 nodules in the liver. These nodules were few in number and the swollen, hemorrhagic appearance was rare. The B mice growing C198a showed almost no tendency for C198a metastasis to the liver or elsewhere.

All the F_1 hybrids of leaden mice developed C198 liver metastases and there was considerable increase in the frequency and size of the nodules over that seen in the pure leaden hosts. The F_1 hybrids of the C57 black strain crossed with stocks other than leaden showed that when C198 was present in the liver it was most frequently expressed as distinct tumor nodules.

TABLE V: RESPONSE OF PULMONARY AND HEPATIC ORGANS TO METASTASES OF C198 IN MICE GROWING SUBCUTANEOUS IMPLANTS OF TUMOR

Strain	No.	Liver Total, % +	Liver S.H., %	Liver Nod., %	Lungs Total, % +	Lungs H %	Lungs Nod., %
L	71	100	96	34	76	76	0
B of (L) — (B)	26	58	4	54	23	23	0
B with C198a	55	2	0	2	0	0	0
F_1 (L × B)	45	100	82	78	91	91	0
F_1 (L × Br^{cd})	42	100	55	57	48	48	0
F_1 (B × Br^{cd})	114	77	51	51	49	47	2
F_1 (B × Br^{a})	46	50	24	46	76	22	65
F_1 (B × dba)	17	18	0	18	12	6	6
F_1 (B × A)	21	30	7	30	11	11	0

S.H. = Swollen and hemorrhagic.
H. = Hemorrhagic.
Nod. = White nodules of C198.

Pulmonary response to C198 metastases.—In the lungs the usual picture of tumor involvement was extensive, hemorrhagic foci without definite tumor nodules. F_1 (B × Br^{a}) hybrids were significantly different from all other types in that C198 metastases formed distinct tumor nodules in the lungs of 65 per cent of the mice autopsied, and most of these were without hemorrhagic foci (Table V). In the case of the F_1 (B × Br^{cd}) mice, 2 had lung metastases and in the F_1 (B × dba) group only 1 mouse had a C198 nodule in its lung.

Organophilic tendencies of C198.—Host mice, one or both of whose parents belonged to the leaden strain, when injected subcutaneously with tumor C198 developed liver metastases in 100 per cent of the cases. When F_1 hybrids of (B × Br^{a}), (B × Br^{cd}) and (L × Br^{cd}) were inoculated with C198 it was found that when one parent was descended from the Br strain the ovaries and kidneys were nearly always strikingly involved by metastases of the tumor.

The F_1 (L × Br^{cd}) and F_1 (B × Br^{cd}) hybrids with implants of C198 were very similar in the gross responses of their livers and lungs to tumor cell emboli. This is compatible with the response of the kidneys and ovaries for the same types of mice, as shown in Table IV.

DISCUSSION

The liver carcinoma, C954, remained localized at the subcutaneous implantation site until the L strain host mouse died from complications brought about by the advanced neoplastic growth. Reticuloendothelioma C198 had a marked tendency to appear in the liver and certain other internal organs of the L strain hosts after their subcutaneous implants of this tumor developed. This was referred to in an earlier paper (1). A similar condition was found in a transplantable reticuloendothelioma, called C800, that arose in a C57 black strain mouse (unpublished data). This tumor also formed metastases to the lungs, spleen and liver. Gorer (4) reported two other transplantable reticuloendotheliomas in C57 black mice. These had like preference for extension, into the liver. When tumor C198 was injected subcutaneously the mass at the implantation site in a leaden host usually remained small, and was soft and doughy. This condition was found also in the case of the three B tumors just referred to.

No published data have been found that describe tumors with as definite organophilic tendencies as have been shown here. These responses vary depending upon the type of host bearing the implants of tumor C198. The mechanical filtering-out of tumor cells by the architectural arrangement of the blood vessels, inherent in certain organs, cannot alone explain this organ-tumor relationship.

After receiving subcutaneous implants of tumor C198 all the leaden strain mice and all their F_1 hybrids showed metastases of this tumor in their livers. In all the inoculated F_1 (L × Br^{cd}) females tumor C198 metastasized to their ovaries, and in both sexes there was kidney involvement in nearly 100 per cent of the animals. On the other hand, only a small percentage of the inoculated F_1 (L × B) hybrids showed C198 tumor nodules in ovaries and kidneys. Metastatic nodules of this tumor have been found in the ovaries and kidneys of leadens but only on rare occasions. These nodules were solitary and scarcely visible. The F_1 (B × Br^{cd}) hybrids reacted to injections of C198 very much the same way as did the F_1 (L × Br^{cd}) mice except that the livers of the former did not always develop metastatic tumor lesions.

The sewing together of a resistant B to a susceptible L mouse in parabiotic union upsets the inherent refractory response of the B host mouse to implants of the L strain tumor C198. Not only did such B mice grow the tumor implants in 91 per cent of the recipients, but 62 per cent of these mice with tumors also developed internal metastases. The internal organs invaded by C198

happened to be the same as those usually involved in the leaden mice that were hosts to the tumor.

The tumor C198 was altered by its growth in the B mice that were living in parabiotic combination at the time of inoculation with this tumor. This altered line of tumor C198 was given the name C198a. B mice inoculated with C198a grew large subcutaneous masses and often lived for 4 to 8 months. However, in the 55 B mice that were hosts to C198a there was but one internal nodule found at autopsy, a tiny liver nodule. Its diagnosis as C198a was confirmed by microscopic examination. The work by Gorer (4) and our unpublished data on C57 black reticuloendothelioma C800 show that the B strain responded to transplants of its own tumors of this type by developing hepatic, pulmonary and splenic metastases. It is possible that the B mice in parabiotic union were more likely to show their inherent response to reticuloendothelioma as a tumor type, but could not as readily show this reaction to a leaden strain tumor placed subcutaneously into B mice that were not in parabiosis.

Two interesting findings are demonstrated hereby. First, the B strain mouse can be altered from its normal refractory response to the leaden neoplasm C198 if the tumor is implanted while the refractory animal is in parabiotic combination with a susceptive mouse. Second, that the growth of this tumor in such a B host, whose physiology has shifted from refractivity to susceptivity to the growth of C198, can alter the physiology of the tumor itself. Thus we can change the host, and the host in turn can change the implanted tumor that it nurtures.

SUMMARY

A carcinoma of liver parenchyma cells and a reticuloendothelioma from the liver were compared as to their organophilic tendencies. This was shown by both the local and the systemic response of the host mice that received subcutaneous injections of fragments of these two tumors. Both neoplasms originated spontaneously in leaden strain mice. Both tumors were implanted and grew successfully at the site of inoculation in all the leaden strain mice tested. Carcinoma C954 never extended beyond the right axillary subcutaneous region where the tumor tissue fragment was deposited. However, the reticuloendothelioma C198 not only appeared at the site of implantation but also metastasized to certain thoracic and abdominal organs. On the basis of this last observation a more detailed study was made of the organs involved by these metastases.

All pure strains of mice, except the leaden strain in which the tumor originated, failed to grow tumor C198 following subcutaneous injections of this tumor. The C57 black strain mice in parabiotic combination with leaden mice underwent a change so that most of these black mice were no longer refractory but became susceptible to implants of this neoplasm. Furthermore, the black strain mice physiologically changed by parabiosis were able to alter tumor C198 while it was growing in them. This physiologically altered tumor could grow in both the black and leaden mouse strain hosts. When the parabiotic black strain mice with subcutaneous growths of C198 were autopsied, 62 per cent of them had metastases to their internal organs. However, metastases to internal organs were found in only 2 per cent of the black strain mice not in parabiosis but growing subcutaneous C198a nodules implanted from the successful growths of C198 in black strain parabiotic hosts.

Several F_1 hybrid classes were made by crossing the leaden and black strains with each other and with several other mouse strains the members of which were refractory to implants of C198. The mice usually grow local masses and those with subcutaneous growths of C198 were autopsied and data were kept upon the appearance of metastatic tumor nodules in their internal organs. The most outstanding finding was that when C198 was transplanted into certain types of mice strong organophilic tendencies were shown by this tumor. Very strong hepatophilic, nephrophilic and ovariophilic tendencies for metastases of C198 were demonstrated in some host types. C198 in the F_1 hybrids from leaden mice crossed with other strains showed an hepatophilic tendency with metastases in 100 per cent of the host hybrids. This was also a characteristic finding in the pure leaden strain mice with C198 implants. No other types showed this involvement of the liver to such a marked degree.

In hybrids with one parent from the brown strain metastases from implants of C198 showed a nephrophilic tendency in 91 to 98 per cent of all mice inoculated with this tumor. Furthermore, 89 to 100 per cent of the females from these same brown strain hybrids developed massive tumor metastases in their ovaries. The other types of mice used showed either only slight or no tendency to develop tumor metastases in their kidneys and ovaries. The F_1 hybrids of Br^a crossed with B, or ($B \times Br^a$), had white tumor nodules in their lungs usually without any accompanying hemorrhage. These were nodules of C198. The characteristic picture in all other types of mice was hemorrhagic

foci in the lungs without grossly visible nodules of tumor when pulmonary metastases of C198 occurs.

CONCLUSIONS

1. The liver carcinoma C954 always remained localized at the subcutaneous site of implantation. The reticuloendothelioma C198 usually appeared concurrently at the implantation site and in one or more of the internal organs of the host mouse.

2. Different types of mice bearing subcutaneous implants of C198 demonstrated diverse organophilic tendencies. A very strong hepatophilic, nephrophilic and ovariophilic affinity was demonstrated by the metastases of tumor C198 in certain types of host mice while other host types showed either irregular or no metastases to their livers, kidneys and ovaries. In many host animals this tumor also showed a tendency to metastasize to the lungs, spleen and lymph nodes.

3. The normal refractory response of B strain mice to C198 implants was changed by parabiotic union of B and L strain animals. In this way it was possible to change the nonsusceptible B host and to make it susceptive to inoculations of tumor C198. Such a B host in turn brought about a physiological change in the implanted tumor and thereafter the B strain mice showed a high degree of susceptibility to this "altered tumor" called C198a.

4. The appearance of tumor metastases within a specific internal organ is probably dependent upon a host-tumor interrelationship rather than upon either the tumor type or the host type alone.

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Sulfhydryl Reduction of Methylene Blue

With Reference to Alterations in Malignant Neoplastic Disease

Maurice M. Black, M. D.

(From the Department of Biochemistry, New York Medical College, New York 29, N. Y., and the Brooklyn Cancer Institute, Brooklyn 9, N. Y.)

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A significant decrease in methylene blue reducing power of plasma from patients with malignant neoplastic disease was previously reported (1). At that time it was suggested that change in a reducing group of the albumin molecule was a likely source of this alteration. Similar conclusions were reported also by Savignac and associates (7) as the result of analogous studies.

In an attempt to evaluate the effect of the sulfhydryl group on the reduction of methylene blue, a study was undertaken with various compounds of known -SH and S-S structures. In addition, an attempt was made to establish a standard method of calibration of various lots of methylene blue, so that more uniform results would be possible in the plasma reducing test.

Glutathione, cysteine hydrochloride and methionine were made up in equimolar solutions (0.0325 M) in distilled water. One cc. of glutathione was added to 0.2 cc. of 0.13 per cent methylene blue in a Wasserman tube. Similarly, 0.2 cc. of methylene blue (0.13 per cent) was added to cysteine and to methionine. The tubes were immersed in a boiling water bath and observed for time of complete decolorization.

The tube containing methionine and methylene blue failed to show any change in color in spite of continued boiling for an hour and a half. On the other hand, complete decolorization was noted in the tubes containing cysteine HCl and glutathione in 6.0 and 15 minutes, respectively.

An attempt was then made to evaluate changes in the reducing time with varying concentrations of cysteine and glutathione. Thus 1 cc. of varying concentrations of cysteine HCl was mixed with 0.2 cc. of methylene blue (0.13 per cent) and the time noted for complete decolorization. Equimolar solutions of glutathione were treated in a similar fashion. The values obtained are indicated in Fig. 1. The results indicate a linear relationship between the cysteine concentration and the reducing power, and a definite limiting value of cysteine concentration for reduction of the methylene blue. The reactions with glutathione are similar, but the reactivity is less than half that of the cysteine. It is noteworthy also that the resultant leuco mixture did not revert back to colored methylene blue on cooling, as was the case with methylene blue reduction by plasma.

Similar relationships were investigated between cysteine and different concentrations of methylene blue. As seen in Fig. 2, similar curves are obtained, but the position of the curve on the graph varies with the concentration of the methylene blue used. It should be noted that there is no appreciable difference in the reducing time of methylene blue on varying the concentrations between 0.10 per cent and 0.2 per cent, although 0.08 per cent shows a decided difference.

Analogous findings were obtained on mixing similar concentrations of methylene blue with a plasma sample. The following reducing times were obtained when a plasma sample was used to reduce the methylene blue solutions:

Methylene blue, per cent	Reducing time, minutes
0.08	5.5
0.13	8
0.20	8

The curves depicted in Fig. 2 possess an additional feature which merits attention in relation to the results obtained with plasma, namely, the sharp break in the curves occurring after the methylene blue reducing time of 15 minutes. A similar break is found at 15 minutes when the occurrence of various reducing times of numerous plasma samples from cancer patients is plotted. These figures are based on 109 plasma samples whose reducing times fell between 13 and 25 minutes. As with the cysteine, apparently, a point is reached beyond which decrease in reducing activity is attended by marked change in the reducing time; in short, a steeper rate of change.

In view of the parallel results obtained with cysteine HCl and plasma, the cysteine solutions may be used to test the reactivity of different lots of methylene blue. Concentrations between 0.1

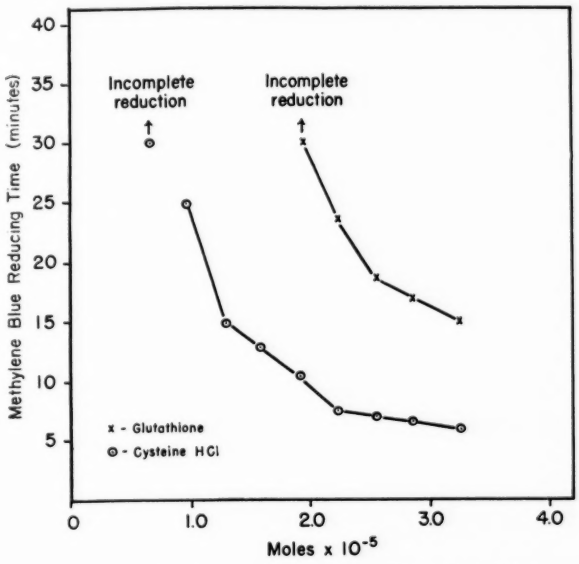

Fig. 1- Reduction of methylene blue (1cc. of 0.13 %) by cysteine HCl and glutathione

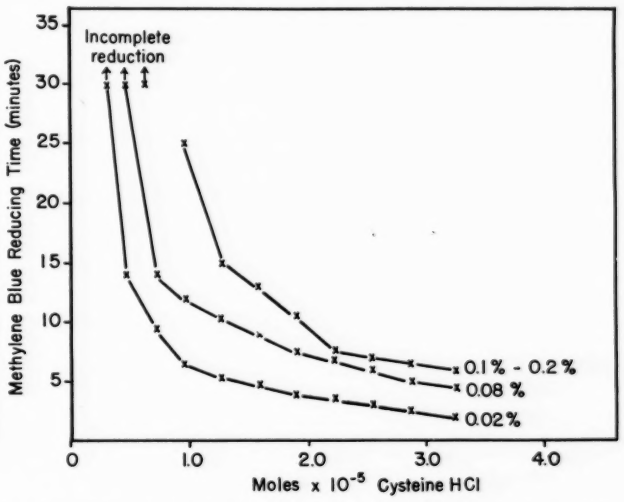

Fig. 2.- Reduction of methylene blue (0.2 cc.) of varied concentration

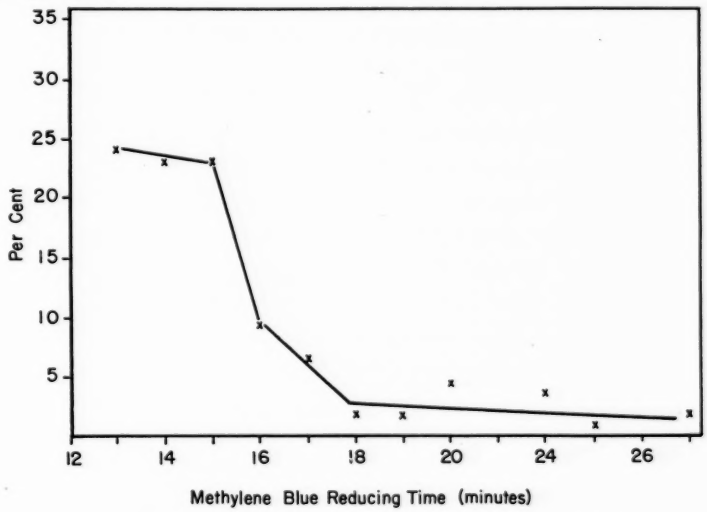

Fig. 3. - Distribution of reducing time values in 109 cancer cases

per cent and 0.2 per cent would seem to be most efficacious, since variations in this zone produce minimal changes in reducing time. Thus, a concentration of 0.13 per cent methylene blue has been adopted in evaluating the reducing power of plasma.

The apparent decrease in sulfhydryl activity in plasma of patients with malignant neoplastic disease suggested therapeutic trial of sulfhydryl compounds on such patients. Glutathione in doses of 50 to 100 mgm. was injected intravenously in several patients with varying types of malignant neoplastic diseases. A reaction of chills and fever occurred in most cases in about 30 minutes and lasted from 20 minutes to an hour. This was usually replaced by a sense of well being and relief from previous symptomatic complaints, *viz.* pain, asthenia etc. Beneficial effects usually lasted several days and could be regained by repeated injections. Similar results were obtained by injection of 25 to 50 mgm. of cysteine HCl although the initial reaction did not occur. It should be mentioned that although the symptomatic improvement might be great, there was no apparent effect on the growth of the neoplasm.

DISCUSSION

The reduction of methylene blue by cysteine and glutathione and not by methionine is an indication that, in these compounds at least, a free -SH bond is required. The more prolonged time found for glutathione (15 minutes) as compared with cysteine HCl (6 minutes) would seem to indicate that the avaiiability or reactivity of the -SH bond may be altered by its location in the molecule. Thus in cysteine the -SH is terminal and presumably unhindered in its reactivity. In

the case of the glutathione, the internal location of the -SH bond seems to decrease its reactivity.

The reversible decrease in reducing power of plasma associated with malignant neoplastic disease might be explained on the basis of changes in the spatial configuration of the albumin molecule. Such changes would be readily reversible and would not necessitate changes in amount of total protein of -SH bonds. This is of importance since the observed decrease in reducing power is not correlated with changes in concentration of plasma proteins.

The observation that glutathione and cysteine were often efficacious in relieving the symptomatic complaints of patients with malignant neoplastic disease finds some parallel in the literature. Thus, Stern and Wilheim (8), in reviewing the relation of sulfhydryl compounds to life processes, point out that the growth inhibition of normal animals treated with various organic carcinogens could be cancelled by increased quantities of sulfhydryl compounds in the diet. This is interpreted as being due to utilization of sulfhydryl compounds in detoxification of the carcinogens and also the competitive utilization of -SH groups by neoplastic tissue itself, leaving relative deficiencies of these indispensible requisites for growth, enzyme activity and metabolism. Therapeutic use of cysteine and glutathione also has been suggested by the observation of tumor growth inhibition in some neoplasms (2, 3, 5), although no such effect was obtained in others (4).

The data presented in this and our previous study would seem to point to the importance of the sulfhydryl group as a factor in the over-all redox substrate of the body rather than as a specific tumor-inhibiting agent. One might consider this group analagous to a buffer in acid-base systems. Loss of such control would lead to suboptimal sulfhydryl potentials with attendant alterations in function of various enzyme systems. The report by Hirshfeld, Duboff, and West (6) of an inhibitory action of serum of cancer patients on the tyrosine-tyrosinase system might be explained on the basis of such alteration in optimal -SH potential.

SUMMARY

1. Methylene blue is reduced by boiling with cysteine HCl and with glutathione but not with methionine.

2. The reaction with cysteine HCl is more rapid (6.0 minutes) than the reaction with equimolar glutathione (15 minutes).

3. The reaction between cysteine and methylene blue shows a linear relationship, which may be used for calibration of different lots of methylene blue.

4. A possible relationship between the spatial configuration of the albumin molecule and the decrease in reducing power of plasma in the presence of malignant neoplastic disease is discussed.

5. Effects of the administration of cysteine and glutathione on patients with malignant neoplastic diseases are reported.

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Abstracts

Reports of Research

Azotoluene Bladder Tumours in Rats. STROMBECK, J. P. [Surg. Clin., Lund, Sweden] *J. Path. & Bact.*, **58**:275-278. 1946.

Azotoluene given by the mouth to rats does not produce neoplastic changes in portions of the bladder transplanted to the liver.—E. L. K.

The Induction of Mammary Carcinoma in "IF" Mice by Cutaneous and Intraperitoneal Administration of Methylcholanthrene. ORR, J. W. [Dept. Exper. Path. and Cancer Research, Univ. of Leeds, Leeds, England] *J. Path. & Bact.*, **58**:589-592. 1946.

The IF strain of mice appears to be resistant to the development of mammary cancer, only one possible mammary carcinoma having been found in an untreated female. On the other hand, fortnightly cutaneous applications of methylcholanthrene dissolved in almond oil induced mammary carcinoma, frequently multiple, in 30 of 37 females of the strain. Tumors developed in all female mice surviving this treatment for 126 days or more, the mean induction time being approximately 140 days. Intraperitoneal injections of methylcholanthrene in sesame oil induced mammary carcinoma in 1 of 12 IF mice after 105 days, the maximum survival period of the group being 113 days.—A. H.

Measurement of the Photodynamic Effect of Cancerogenic Substances with Biological Indicators. MATOLTSY, G., and FÁBIÁN, Gy. [Biol. Research Inst., Tihany, Hungary] *Nature*, **158**:877. 1946.

When *Drosophila* flies were fed in the dark on a standard diet containing carcinogens (1 mgm. per 13 gm.), the hydrocarbon could be detected in the organs and cells of of the larvae by fluorescence microscopy. The survival time after raying with ultraviolet light was found to be as follows: benzpyrene treated for 6 minutes 30 seconds; methylcholanthrene 13 minutes 35 seconds; dibenzanthracene 19 minutes 34 seconds; controls *i.e.* larvae bred on standard diet, survived 39 minutes 51 seconds. (No mention is made of controls which should have been made where fluorescent noncarcinogenic hydrocarbons were added to the diet.)—I. H.

Test of a Cancerogenic Substance in Respect to the "Non-disjunction" Frequency of the X-Chromosomes in Drosophila. FÁBIÁN, Gy., and MATOLTSY, G. [Biol. Research Inst., Tihany, Hungary] *Nature*, **158**:911-912. 1946.

Drosophila larvae and adult flies were fed on a diet containing benzpyrene, and the hydrocarbon could be detected by fluorescence microscopy in the larvae, eggs and ovaries. Some tests have been made on the effect of carcinogenic substances on *Drosophila* in respect to mutation frequency (Auerbach, C., *Proc. Roy. Soc. Edinburgh*, **60**:164; Friedrich-Freksa, H., *Biol. Zentralbl.*, **60**: 498). The results showed that the mutation rate does not increase following applications of carcinogenic chemicals. The cultures were kept in darkness, while the primary nondisjunction was investigated. Bar males and white females were used from 2 inbred stocks which had been kept pure for 5 years, and so may be considered well-balanced stocks from the point of view of modifying factors. In this standard arrangement the frequency of exceptions was 1:500, or 0.2 ± 0.05% without benzpyrene. There was not a significant difference between the control and the treated cultures in respect to the number of exceptional offspring. In a succeeding test, the adult females were fed entirely on food containing benzpyrene, and in this case, using the fluorescence microscope, it was evident that the benzpyrene was also present in the eggs when they were laid. In this test the ratio of exceptions was 1:2,300, or 0.04 ± 0.03%. The standard errors showed a statistically significant difference (0.16 ± 0.059) in the negative direction between controls and the second test. None of the treatments used gave any detectable increase in the nondisjunction frequency; but treatment with benzpyrene decreased the number of exceptional flies, that is, the mutation rate. The reason for this is not known.—I.H.

Uncoordinated Growth in Paramecium Induced by "Gammexane." LLOYD, L. [Univ. of Leeds, Leeds, England] *Nature*, **159**:135. 1947.

The γ-isomer of hexachlorocyclohexane produces in *Paramecium* large multinucleated forms, as the late J. C. Mottram observed, after treating these organisms with carcinogenic hydrocarbons.—E. L. K.

Induction of Glandular Carcinomas of the Prostate in the Mouse. HORNING, E. S. [Imperial Cancer Research Fund, London, England] *Lancet*, **2**:829-830. 1946.

The epithelium from either the anterior or dorsal lobes of the prostate of 6 months old Strong A mice was cut into strips, wrapped around some crystals of 20-methylcholanthrene, and implanted subcutaneously in male mice of the same strain. Three such grafts can be made in one host. In 10½ weeks small tumors appear; of 11 tumors 10 were glandular and 1 squamous cell in nature. Some are growing in the eighth grafted generation, and show considerable secretory activity.—E. L. K.

Significance of Carcinogenic Agents. ŠULA, J. *Časop. lék. česk*, **80**:698-700. 1941.

The author discussed the similarity of the chemical structure of methylcholanthrene and bile acids and the

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possibility of the production of methylcholanthrene from some products of the sterol metabolism in the organism. The relationship to the sex hormones is also considered. —B. S.

Relations of Steroid Hormones and Anhydro-Hydroxy-Progesterone to Fibromatosis. IGLESIAS, R., and LIPSCHÜTZ, A. [Nat. Health Service and Univ. of Chile, Santiago, Chile] *Lancet*, **2**:488-490. 1946.

Anhydro-hydroxy-progesterone has an inhibitory action upon uterine fibroids, and to a lesser extent upon other abdominal fibroids, induced in castrated female guinea-pigs by 2-estradiol. No masculinizing action was observed. The authors suggest clinical trials of this compound, together with small quantities of testosterone propionate, in cases of uterine fibroids.—E. L. K.

Genes and Nucleoproteins in the Synthesis of Enzymes. SPIEGELMAN, S., and KAMEN, M. D. [Mallinckrodt Inst. of Radiol., and Washington Univ. Sch. of Med., St. Louis, Mo.] *Science*, **104**:581-584. 1946.

Yeast cells were grown in a medium containing radioactive phosphorus (P^{32}) for 48 hours, at which time all fractions contained P^{32} in equilibrium with the P^{32} in the medium. The cells were then washed, and were allowed to ferment glucose anaerobically in absence of nitrogen. Under these conditions no protein synthesis or cell division occurred, and there was no loss of P^{32} from the nucleoprotein fraction of the cells even though the non-nucleoprotein, acid soluble phosphorus was rapidly equilibrated with that of the medium. The same result was obtained in the presence of nitrogen when cell division was inhibited by sodium azide or dinitrophenol. When, however, the cells were allowed to synthezise protein and to divide, it was found that large amounts of P^{32} were lost from the nucleoprotein, indicating a flow of phosphate from this fraction in the course of synthesis. The same flow of P^{32} from nucleoprotein was observed when the cells were induced to form a new enzyme in the course of adaptation to a new substrate.

These results plus other evidence reviewed in this paper have led the authors to suggest that nucleoproteins are the controlling elements in protein formation. They hypothesize that genes continually give off at different rates partial replicas of themselves to the cytoplasm. These replicas are nucleoprotein in nature, possess in varying degrees the capacity for self reproduction, and together determine the types and amounts of proteins and enzymes synthesized. Furthermore, it is supposed that a competitive balance exists among the various cytoplasmic nucleoproteins, that this balance may be changed by changing certain conditions under which the cells live, and that such altered relationships among the cytoplasmic nucleoproteins may be passed on to subsequent cell generations. This theory thus attempts to bring together under a unified interpretation (1) Mendelian, or "gene" controlled inheritance, (2) cytoplasmic inheritance, (3) cellular differentiation, and (4) enzymatic adaptation. The origin of cancer as a sudden heritable change in somatic cells, analogous in many ways to enzyme adaptation or cellular differentiation, also comes within the scope of this theory.—R. B.

Ultra-Violet Absorption in Living and Dead Cells. BRUMBERG, E. M., and LARIONOW, L. TH. [Optical Inst., and Central Roentgenological, Radiological and Cancer Inst., Leningrad, U. S. S. R.] *Nature*, **158**:633-664. 1946.

An ultraviolet microscope equipped with a special achromatic objective (aperture 0.5) was employed for photographing living tissue cultures. The source of light was a high-pressure quartz mercury lamp. All radiations except of wave length 254-275 $\mu\mu$ were eliminated by filters. Focussing was performed by ordinary lighting, so that no ultraviolet rays reached the cells prior to their being photographed. When living mouse mammary carcinoma cells and mouse and chick fibroblasts were examined, only nucleoli and the cytoplasm of cancer cells revealed moderate absorption in the region 254-275 $\mu\mu$. The cytoplasm of cancer cells vitally stained with neutral red lost its capacity for absorption. Preliminary exposure of cultures to direct ultraviolet irradiation (without filters) for 2 minutes resulted in definite cytological changes. Nuclei then acquired the property of absorption and photographs presented the same appearance as those published by Caspersson.

It is suggested that desoxyribonucleic acid is contained in the nuclei of living cells in a form which does not absorb ultraviolet rays of wave length about 260 $\mu\mu$, and that absorption only occurs in injured and dead cells.—R. J. L.

Studies in Vitro on Cellular Physiology. The Effect of X-rays on the Survival of Cells. SCHREK, R. [Veterans Administration, Hines, Ill.] *Radiology*, **46**:395-410. 1946.

Cellular suspensions of thymus, spleen, bone marrow, and testes of rabbits and from leukocytes of normal and leukemic blood of men were irradiated with 20 to 5,000 r and incubated at 37° C. for 1 to 7 days. Periodic examination of cell counts and stained smears were made and graphed as to the 50 and 10 % survival time. A dose of 1,000 r to thymic and splenic suspensions produced no perceptible change in 3 hours but produced a relatively rapid decrease in the unstained cell counts after this period. Irradiated leukocytes from normal and lymphocytic blood had a shorter survival time than non-irradiated blood. Suspensions from myelogenous leukemia, bone marrow and testes showed no effect of irradiation. Tests made under anaerobic conditions showed no perceptible decrease in the number of eosin-resistant lymphocytes which had been irradiated.—R. E. S.

Mechanism of Radiation Effects Against Malignant Tumors. WARREN, S. [Boston, Mass.] *J. A. M. A.*, **133**: 462-463. 1947.

It is the radiant energy absorbed by the tissue or cell that is effective, not that which is delivered to it. The first noticeable effect on cells is interference with mitosis —both diminution in mitotic activity and the appearance of chromosomal abnormalities followed by vacuolization and swelling of the cytoplasm. There are concomitant effects produced on the connective tissue stroma and blood vessels within the irradiated area. The vascular endothelium is damaged inducing thrombosis. Hence local impairment of circulation in the tumor and tumor bed results. Hyaline changes leading to the formation of dense

collagen thus act as an added barrier to the metabolic activities of tumor cells and hinders their spread to adjacent structures. The same general order of sensitivity prevails among the tissues exposed to radiation by an atomic blast as by therapeutic means.—M. E. H.

Biochemical Aspects of Over-Activity of the Adrenal Cortex. SCOWEN, E. F., and WARREN, F. L., *Proc. Roy. Soc. Med.*, **40**:39-43. 1946

Adrenal cortical carcinoma in females of all ages is associated, except in rare cases, with increased excretion of 17-ketosteroids; an excessive excretion of dehydroisoandrosterone is characteristic of these tumors. Owing to the rarity of adrenal carcinoma in males it is not yet possible to say whether the sterol metabolism is the same in them as in female patients.—E. L. K.

The Disposition of C^{14} in Bone. BLOOM, W., CURTIS, H. J., and MCLEAN, F. C. [Univ. of Chicago, Chicago, Ill., and Monsanto Chemical Co., Dayton, Ohio] *Science*, **105**: 45. 1947.

C^{14} injected as carbonate into rats was deposited in bone primarily in non-growing areas, and persisted there apparently undiminished for the 4 months' duration of the experiment. In contrast, the C^{14} deposited in the liver and kidney remained there for only 2 weeks. The authors suggest that the health hazards involved in working with this radio-isotope of carbon should be studied with special reference to the possible development of bone tumors. —R. B.

A Plan for Analysis of the Biologic Factors Involved in Experimental Carcinogenesis of the Thyroid by Means of Radioactive Isotopes. HERTZ, S. [Boston, Mass.] *West. J. Surg.* **54**:487-489. 1946.

A plan is presented for the analysis of experimental carcinogenesis in animals, drawn up from experience to date, from the study of normal and pathologic thyroid physiology, chemistry and therapeutics by means of radioactive isotopes of iodine. It is hoped that a logical theory of carcinogenesis and an understanding of important preventive and therapeutic factors may be evolved.—M. E. H.

The Effect of Certain Azo Dyes upon the Storage of Riboflavin in the Liver. GRIFFIN, A. C., and BAUMANN, C. A. [Univ. of Wisconsin Coll. of Agric., Madison, Wis.] *Arch. Biochem.*, **11**:467-476. 1946.

Since riboflavin is known to counteract the carcinogenicity of several of the azo dyes, investigations were carried out to determine whether the effect of a given dye on hepatic riboflavin might parallel its potency in inducing liver tumors. Such a parallelism was observed. The addition of many carcinogenic azo dyes to the diet of rats caused some decrease in the riboflavin content of the liver. The decrease appeared to be roughly equivalent to the carcinogenicity of the dye: *m'*-methyl-*p*-dimethylaminoazobenzene was most effective, *p*-dimethylaminoazobenzene and *p*-monomethylaminoazobenzene were fairly effective, whereas *o'*-methyl-*p*-dimethylaminoazobenzene, *p'*-methyl-*p*-dimethylaminoazobenzene, aminoazotoluene, aminoazobenzene, or azobenzene had little or no effect.

More riboflavin was stored in the liver when the basal diet contained 24% of casein than when 12% was fed. The relative effects of the carcinogens were essentially the same on both diets.

The presence of carcinogenic azo dyes in the diet resulted in a decreased food intake, but this was not responsible for the impaired vitamin storage. Rats fed restricted amounts of the control diet free from the dye showed only a slight decrease in the total amount of riboflavin per liver while the concentration of vitamin per gm. of liver tissue was usually higher than in rats fed *ad libitum*.—Authors' abstract.

The Influence of Liver L. casei Factor on Spontaneous Breast Cancer in Mice. LEWISOHN, R., LEUCHTENBERGER, C., LEUCHTENBERGER, R., and KERESZTESY, J. C. [Mount Sinai Hosp., New York, N. Y.] *Science*, **104**:436. 1946.

Among 28 Rockland mice injected intravenously daily with 5 μgm. of fermentation *L. casei* factor over a period of 4 to 6 weeks there were 11 in which the mammary cancer regressed completely; after 100 days 23 mice were still alive. Among 39 mice injected in the same way with a daily dose of 5 μgm. of liver *L. casei* factor there was only 1 complete regression, and the mean life span was 75 days. Lung metastases in this group were more numerous than among the controls. Thirty-one additional mice were treated with daily doses of 100 μgm. of liver *L. casei* factor. In this group there were no regressions; in fact the mammary tumors grew faster than did those of the controls. The mean life span was 55 days. Among the 71 control mice there were no regressions and the mean life span was 74 days.—R. B.

Les facteurs antiblastiques d'origine alimentaire. [The Anti-Blastic Factors of Dietary Origin.] MAISIN, J., and POURBAIX, Y., *Bull. Assoc. franç. p. l'étude du cancer*, **29**:223-251. 1940.

A review with 31 references is presented. Protocols are presented of 13 experiments, previously reported by Maisin and his colleagues, showing the cancer-inhibiting action of various foods, including fresh beef heart, whole rye flour, and fresh baker's yeast.—G. H. H.

Tumors in Intrasplenic Ovarian Transplants in Castrated Mice. LI, M. H., and GARDNER, W. U. [Yale Univ. Sch. of Med., New Haven, Conn] *Science*, **105**:13-15. 1947.

Ovaries grafted into the spleens of castrated mice (A strain, C_3H strain, $C_3H \times A$ F_1 hybrids) developed tumors 130 to 346 days after grafting. Among 21 castrated males there developed 5 (possibly 7) granulosa cell tumors plus 1 mixed tumor. Among 33 castrated females, in which the intrasplenic ovarian grafts showed no adhesions to adjacent structures, there developed 4 luteomas plus 7 mixed tumors. Among 19 additional castrated females, in all of which the intrasplenic ovarian grafts showed vascularized adhesions, there developed only 1 luteoma in a mouse that had irregular estrus cycles during the latter part of the experimental period. No tumors developed from control grafts of ovaries into

the subcutaneous tissue of castrated females, normal and castrated males, and into the testes of normal males; except for one questionable granulosa cell tumor developing from a subcutaneously transplanted ovary in a castrated female mouse.

The development of tumors from intrasplenic ovarian grafts appears to be due to an increased production of gonadotropins in the host following castration. Grafts placed in the spleen are exposed to these hormones before they reach the liver where they appear to be inactivated. —R. B.

Hypervolemia in Mice Bearing Transplantable Granulosa Cell Tumors. FURTH, J., and SOBEL, H. [Cornell Univ. Med. Coll., and New York Hosp., New York, N. Y.] *Science*, **105**:41. 1947.

Mice bearing transplanted granulosa cell tumors were previously found to have livers and other abdominal organs congested with blood. In the present study these animals were shown to have striking increases in blood volume. By the exsanguination-perfusion technic, the blood volume, in per cent of body weight, was found to be 13.6 for the experimental mice compared with 5.2 for the controls. By the Evans blue (T-1824) dye technic, the values were 34.3 and 10.9 respectively. The hematocrit values of the blood of the experimental mice were normal, indicating a large increase in both plasma and red cells. Control mice bearing any of four other types of tumors showed no increase in blood volume.

It is suggested that the granulosa cell tumors may secrete a substance causing the hypervolemia, and that this may be accompanied by the appearance of excessive amounts of a vaso-depressor material which would account for the observed vasodilation associated with the blood volume increase.—R. B.

The "Cytogenetics" of Black and White Guinea Pig Skin. BOILLINGHAM, R. E., and MEDAWAR, P. B. [Univ. of Oxford, Oxford, England] *Nature*, **159**:115-117. 1947.

Black guinea pig epidermis "infects" and thus blackens white epidermis upon which it is grafted. This is due, not to invasion and displacement of white cells by black, but to some agent which enters the white cells and brings about a permanent heritable change that causes them and their descendents to become and remain black.—F. L. K.

Interpretive Morphology. MOORE, R. A. [Washington Univ. Sch. of Med., St. Louis, Mo.] *Proc. Inst. Med. Chicago*, **16**:306-312. 1947.

The theme of the lecture was that pathologic anatomy can take a place beside experimental pathology as a dynamic science. In the mind of the observer of morphologic changes, there may be the consideration of the contraction of muscles, the secretion by cells, the action of enzymes, and the elaboration effects of hormones, as well as parthogenetic development of the sex cell. Examples of the histogenesis and development of various tumors of the genitourinary tract served as examples.—M. E. H.

General Pathology of Tumors of Endocrine Glands. KARSNER, H. T. [Western Reserve Univ. Sch. of Med., Cleveland, Ohio] *Bull. New York Acad. Med*, **22**:503-510. 1946.

A general review of tumors of endocrine glands including brief discussions of the genesis, morphology, chemical components and physiological manifestations of these tumors.—M. T.

Protective Action of Desoxycorticosterone Acetate Against X-Ray Induced Liver Changes. ELLINGER, F. [Long Island Coll. of Med., Brooklyn, N. Y.] *Science*, **104**: 502-503. 1946.

This paper reports a study of desoxycorticosterone acetate as a remedy for radiation sickness, this sterone being selected because it counteracts effects of histamine or histamine-like substances which may be the cause of radiation sickness.

One hundred and sixty-eight male white mice were given 500 and 1,000 r/air in one exposure or in fractions of 100 r daily. Seventy-nine of these animals were given in addition daily subcutaneous injections of 0.25 or 0.50 mgm. desoxycorticosterone acetate (in oil), the total dose varying between 2.5 and 8.0 mgm. The most striking effect of the sterone was a reduction in the amount of sudanophile fat appearing in the liver as a result of the irradiation. There was also a slight decrease in mortality among the injected animals, but no definite alteration in the radiation effects on spleen and bone marrow.—R. B.

A New Method of Making Radon Ointment. CARDENAS, L., and WEATHERWAX, J. L. [Philadelphia Gen. Hosp., Philadelphia, Pa.] *Radiology*, **46**:381-384. 1946.

A method of making radon ointment by impregnating charcoal or other adsorbent with radon is described.—R. E. S.

Les tumeurs mammaires bénignes chez l'animal. Leur intérêt biologique. [Benign Mammary Tumors of Animals. Their Biological Aspect.] ROUSSY, G., and GUÉRIN, M. [Cancer Inst., Paris, France] *Presse méd.*, **52**:313-314. 1944.

A review.—C. A.

Some Notes on the Cancer Problem. KOSOLAPOFF, G. M. [Monsanto Chemical Co., Dayton, Ohio] *Science* **104**:491-492. 1946.

In a preceding letter to *Science* (**104**:167. 1946.) K. S. Pilcher proposed that the cancer problem should be attacked through a large-scale, well planned, completely co-ordinated program directed by a group of experts following, as examples, the successful wartime programs in atomic physics, penicillin production, and so on. In the present letter Kosolapoff points out that the successful wartime programs were based on discoveries already in existence, which indicated a clearly defined line of approach. No such clear-cut line of approach to a solution of the cancer problem can be said to exist at present. Therefore, while promising fields of investigation now known should be supported in co-ordinated research, there should be at least equal support given to independent groups of investigators not tied to any definite approach.—R. B.

Clinical and Pathological Reports

Clinical investigations are sometimes included under Reports of Research

Diagnosis—General

The Roen'gen Diagnosis of Pancreatic Cyst. Holt, J. F. [Univ. of Michigan, Ann Arbor, Mich.] *Radiology*, **46**:329-333. 1946.

A correlation of clinical and roentgen findings will often enable the roentgenologist to diagnose pancreatic cyst, particularly cysts arising in the tail of the pancreas. In such cases anterior displacement of the stomach and a smoothly rounded indentation relatively high on the greater curvature of the stomach associated with a rounded, ballotable, freely movable mass in the left upper quadrant of the abdomen permit a reasonable assurance of a correct diagnosis.—R. E. S.

The Vaginal Smear. Practical Applications in the Diagnosis of Cancer of the Uterus. Meigs, J. V. [Vincent Memorial Hosp., Boston, Mass.] *J. A. M. A.*, **133**:75-78. 1947.

The diagnosis of cancer of the cervix as well as cancer of the endometrium can be made with a high percentage of accuracy by the vaginal smear method. It is of tremendous value in the routine screening of patients either in the clinic or in the hospital. Early cases of cancer, *i.e.* "cancer *in situ*," can be diagnosed by this method. The positive vaginal smear indicates the necessity of further examination as well as the need for biopsy specimens. In this way the pathologist may confirm the observation disclosed by the smear. Although smears obtained from the vagina may be an office procedure, the interpertation of the sample should be made by a trained cytologist, one familiar with this method of diagnosis.—M. E. H.

Therapy—General

Treatment of Basal Cell Epithelioma by Injection of Tissue Extracts. A Preliminary Report. Amersbach, J. C., Walter, E. M., and Sperti, G. S. [New York Post-Graduate Med. Sch. and Hosp., New York, N. Y.] *Arch. Dermat. & Syph.*, **54**:119-132. 1946.

A brief review is presented of literature pertinent to the treatment of cancer by non-destructive methods in which tissue extracts are used to induce tumor resistance in laboratory animals. The close relationship between normal and cancer cell metabolism and the resistant state is pointed out. In this series 21 patients with basal cell epitheliomas were treated with intradermal injections of spleen or liver extracts. Fourteen patients showed complete regression of the lesions, in 1 no improvement was seen, and the remainder, still under observation, were steadily improving.—H. H.

Radiation Therapy Conference. Cantril, S. T., and Buschke, F., Editors. [Swedish Hosp. Seattle, Wash.] *West. J. Surg.*, **54**:369-370. 1946.

The largest palliative accomplishment of roentgen therapy in breast cancer is in the retardation of bone metastases and the alleviation of pain which it gives.—M. E.H.

Modern Developments in Radiotherapy and Their Practical Applications. Charteris, A., and Park, S. D. S., *Glasgow, M. J., Trans. Roy. Med. & Chir. Soc. of Glasgow*, **24**:35-45. 1944.

A review.—E. L. K.

Organisation of Cancer Treatment. *Brit. M. J.*, **1**:921-922. 1946.

The 16th Annual report of the National Radium Trust and Radium Commission covers the period 1944 to 1945 and contains hitherto unpublished observations in the annual reports of the war years. By the authority of the Cancer Act (1939) the commission has been increased from 10 to 17 members and is now in charge of radioactive materials other than x-rays and radium. Moreover, the Commission has advised local authorities with regard to organisation of cancer services. It was recommended that radiotherapy should be carried out only by experienced persons; that physicists should be an integral part of the staffs; and that radiotherapy centers should be limited in number and possess suitable apparatus and personnel to serve a population of 2 million. The Commission has also drawn up a system of records which is now in use for cancer patients.—M. L.

Radiation

The Treatment of Late Post-Irradition Ulcers with Radon Ointment. Low-Beer, B. V. A., and Stone, R. S. [San Francisco, Calif.] *Radiology*, **46**:149-158. 1946.

Twenty-eight patients with post-radiation ulcers were treated with radon ointment containing 36 μc. of radon per gm. of vaseline. Details of the technic for its use are given. Alleviation of pain and healing of the ulcers occurred when there was no extension of the ulcer to bone and no malignant change. The authors consider radon ointment the preferable nonsurgical method of treatment of postirradiation ulcer.—R. E. S.

Energy Absorption in the Trunk in the Radium Treatment of Breast Cancer by Interstitial and Surface Applicator Methods. Wilson, C. W. [Westminster Hosp., London, England] *Radiology*, **46**:364-372. 1946.

The integral dose, *i.e.*, the total energy absorbed in the trunk, has been calculated for conditions corresponding to those of interstitial and surface radium therapy of cancer of the breast. Graphs were then made showing: (1) variation of integral dose per mgm. hr. with positions of radium along the trunk; (2) variation with distance of radium beyond the trunk; and (3) variation with distance from the axis of the trunk, and integral dose for surface applicators. Correlation of integral dose with reaction of lymphocytes during interstitial radium therapy of cancer

of the breast indicates a regular behavior. It is felt that site and dose rate are important factors in assessing the clinical importance of integral dose.—R. E. S.

Protection Measurements of Lead-Shielded Radium. Braestrup, C. B. [Dept. of Hosps., New York, N. Y.] *Radiology*, **46**:385-390. 1946.

Most protection measurements are based on studies obtained with narrow collimated beams but in most instances scattered radiation from neighboring objects is an important contributing factor to the dose received by radium workers. This is borne out by measurements made with the narrow beam and wide beam. Scattered radiation may add materially to the calculated dosage rate. Lead protection shields also depend on the arrangement of the walls and shape of the shield. Better protection may be obtained by limiting the cross-section of the beam or by locating the radium at a distance from scattering objects.—R. E. S.

Skin and Subcutaneous Tissues

Keloid Formation in Both Ear Lobes. Weaver, D. F. [Detroit, Mich.] *Arch. Otolarying.*, **44**:212-213. 1946.

The recurrence in a Negro woman of large keloids, which developed after the ear lobes were pierced for the insertion of earrings, were apparently prevented by roentgen rays.—C. R. N.

Adenomyoepithelioma of Sweat Gland. Report of a Case. Hartz, P. H. [Public Health Service, Curaçao, N. W. I.] *Am. J. Clin. Path.*, **16**:385-390. 1946.

A case report.—S. H. D.

Small Tumours of the Skin. MacKechnie, H. A. [Vancouver, B. C.] *Canad. M. A. J.*, **56**:56-58. 1947.

The author discusses tumors of the skin both in the neoplastic sense and those resulting from some inflammatory process giving rise to hyperplasia or hypertrophy of tissue. All lesions suspected of malignancy should be subject to a biopsy for confirmation of the diagnosis.—M. E. H.

Tumors of the Skin. A Review of Recent Literature. Part I and Part II. Beerman, H. [Philadelphia, Pa.] *Am. J. M. Sc.*, **211**:480-504, and **212**:479-505. 1946.

A full review of the subject with 4 pages of references.—M. T.

Cancer of the Skin. A Statistical Report. Ullmann, H. J. [Santa Barbara, Calif.] *Radiology*, **46**:279-281. 1946.

During a period of 23 years the author saw 1,347 cancers of the skin, treating 1,269 by radiation and 78 by surgery. Four hundred and forty-three of those treated by radiation were followed 2 or more years and showed 25 recurrences. No treatment factors are included in this statistical report.—R. E. S.

Nervous System

A Preliminary Report of the Study of 200 Autopsy Cases at the Eastern State Hospital with Special Emphasis on Neuropathology and Brain Tumor in Old Age. Zfass, I. S., and Riese, W. [Eastern State Hosp., Williamsburg, and Med. Coll. of Va., Richmond, Va.] *Virginia M. Monthly*, **71**:281-287. 1944.

The report concerns itself with 205 patients autopsied during the 5 year period 1937 to 1942. Among these 65% were over 60 years of age and 83% over 50. There were 10 (4.9%) neoplasms among the 205 autopsies; 7 occurred in patients past 50 and 4 of the 7 were 64, 70, 71 and 76 years of age. Histological examination revealed 3 with glioblastoma multiforme, 3 tuberculomas and 1 each with spongioneuroblastoma, meningioma, adenoma of the pituitary (chromophobe) and 1 unclassified tumor. Three case histories are briefly recorded. The authors note that in the aged, the clinical history of tumor may be of short duration and the number of classical symptoms small or entirely lacking.—M. E. H.

Lesions of the Aqueduct of Sylvius. Wilson, H., and Lutz, W. G. [Yale Univ. Sch. of Med., New Haven, Conn.] *Radiology*, **46**:132-138. 1946.

The roentgen demonstration of the occlusion of the aqueduct of Sylvius is often difficult. Where conventional views fail to demonstrate the aqueduct, laminography may give clear visualization. Localization of the site of obstruction is very important. A group of cases of obstructive hydrocephalus was studied by the authors, 11 of which were produced by neoplastic stenosis of the aqueduct and 3 were due to non-neoplastic stenosis. A new roentgen sign for differentiation of supratentorial and infratentorial lesions is described. This is based on the measured angle included between a line drawn along the base of the anterior fossa and a second line drawn from the anterior clinoid process to the suprapineal recess. The theories of pathogenesis of non-neoplastic stenosis of the aqueduct are discussed.—R. E. S.

Schwannoma of the Pharynx with Paralysis of the Vocal Cord. Turchik, F. [St. Vincent's Hosp., Bridgeport, Conn.] *Arch. Otolaryng.*, **44**:568-573. 1946.

A description is presented of a Schwannoma located in the right vagus nerve at its exit from the jugular foramen. Among the symptoms observed were unilateral paralysis of the vocal cord, Horner's syndrome, weakening of the trapezius muscle and progressive involvement of the trunk, pharyngeal and palatal musculature.—C. R. N.

Neurinoma of the Facial Nerve. Kettel, K. [Hilleröd, Denmark] *Arch. Otolaryng.*, **44**:253-260. 1946.

This paper adds another facial neurinoma to the 16 previously reported in the literature. It was located in the pars descendens of the facial nerve of a 32 year old woman. The question is whether it is correct to regard the facial neurinomas as examples of neurinomas originating from an unmixed motor cranial nerve. As several authors have maintained that the seventh nerve contains

some sensory fibers, and as neurinomas practically always arise from sensory nerves or from sensory roots of mixed nerves, this tumor might possibly have arisen from Schwann cells of sensory fibers. Also suggestive of this explanation is the fact that the patient suffered severe facial pain for several years. The literature is reviewed. —C. R. N.

Breast

The Value of Orchiectomy in the Treatment of Carcinoma of the Male Breast. Leucutia, T. [Harper Hosp., Detroit, Mich.] *Radiology*, **46**:441-447. 1946.

Two cases of advanced carcinoma of the male breast were submitted to orchiectomy. In the first case in which there was extensive osseous metastases there was marked improvement and the patient was well 2 years after operation. In the second case there was local recurrence and liver metastasis. A temporary improvement was obtained but within a few months the patient succumbed to widespread visceral disease. These cases suggest that osseous metastases can be brought under control by castration whereas local recurrence and visceral metastases are influenced little or not at all. This conforms with present experiences with castration in mammary carcinoma of the female.—R. E. S.

Surgery of the Breast: A Review. Burton, J. A. G. [Univ. of Glasgow, Glasgow, Scotland] *Glasgow M. J.*, **27**:345-354. 1946.

This review deals with malformations, injuries, inflammatory conditions, and tumors of the breast.—E. L. K.

Paget's Disease of the Nipple. With Special Reference to the changes in the Ducts. Inglis, K. [Univ. of Sydney, Sidney, N. S. W., Australia] *Am. J. Path.*, **22**:1-33. 1946.

Two cases are reported and an account is given of the changes that occur in Paget's disease. Evidence is submitted in support of the opinion that the epidermal lesion of the nipple precedes the infiltrating cancer in the underlying breast and is linked to this infiltrating cancer by spreading down one or more ducts by the intraepithelial route.—J. G. K.

Papillomata of the Breast. Wakeley, C. [King's Coll. Hosp., London, England] *Lancet*, **1**:62. 1947.

Intracystic papilloma was the cause of a blood-stained discharge from the nipple in 62 of 119 patients treated by the author. Local excision of the tumor and not local excision of the breast is the best treatment. Follow-up records of 45 of the 62 cases show that in not one case has there been any malignant disease after local excision. X-ray and radium therapy are useless. The age incidence is from 20 to 60 years with a maximum between 40 and 45 years. Most of the tumors in the author's series were near the nipple, but 5% were at the periphery of the breast and one was in the axillary portion.—E. L. K.

Diagnosis and Treatment of Carcinoma of the Female Breast. Oberhelman, H. A. [Loyola Univ. Sch. of Med., Chicago, Ill.] *S. Clin. North America*, **26**:116-129. 1946.

In the diagnosis and treatment of breast cancer, the value of early recognition and early surgery is duly stressed. The author believes that every breast "lump" should uncompromisingly be submitted to biopsy. The diagnostic procedures of aspiration, transillumination and radiography should be looked upon as having limited value as accurate diagnostic procedure, and they are not practical for routine breast examinations.

The employment of methods of treatment, such as castration and sex hormone therapy, should be regarded as palliative and not curative. The method of choice in combating cancer of the breast is meticulous radical or modified surgery, combined with irradiation therapy.—J. L. M.

Female Genital Tract

Dysgerminoma of the Ovary with Widespread Metastases. Pendergrass, E. P., and Selman, J. [Univ. of Pennsylvania Hosp., Philadelphia, Pa.] *Radiology*, **46**: 377-379. 1946.

A case is reported of dysgerminoma of the ovaries in a child whose first symptom was an orbital tumor.—R. E. S.

[Dermoid Cyst of Ovary, with Torsion and Infarction.] Case Records of the Massachusetts Gen. Hosp. Case 32362. *New England J. Med.*, **235**:340-341. 1946.

A report of a case.—M. H. P.

Carcinoma of the Fundus Uteri. Crossen, J. R. [St. Louis, Mo.] *South. M. J.*, **39**:445-452. 1946.

The problem and evaluation of present therapeutic methods is discussed, and the use of a flexible wire distributor for placing radium preoperatively in the uterine cavity is described.—W. A. B.

Inspissated Blood and The Growth of Fibromatous Uterine Tumors. Marshall, W., Holloway, A. L., Irby, L. E., and Peacock, C. [Mobile, Ala.] *Am. J. Surg.*, **72**: 57-62. 1946.

The theory of an active chemotropism operating in the process of fibromatosis, as advanced in a previous article by Marshall, is that serum extravasated in tissue draws fibroblasts into that area. This concept is suggested as explaining the etiology of uterine fibroids. A preliminary experiment in which an attempt was made to inject whole blood into the uterine horn of a 6 months old female puppy, failed to demonstrate any changes in the uterine horn 2 months after the injection.—W. A. B.

[Endometriosis.] Case Records of the Massachusetts Gen. Hosp. Case 32482. *New England J. Med.*, **235**: 801-803. 1946.

This is the report of a case in which endometriosis involved the fragments of 2 ovaries that remained after the removal of an ovarian cyst, the posterior surface of the uterus, and probably the wall of the sigmoid.—M. H. P.

Endometriosis and Adenomyosis. Yin, Y. C. [National Central M. S., Chengtu, China] *West J. Surg.*, **54**:490-493. 1946.

The author differentiates between adenomyosis (intrauterine aberrant endometrial proliferations) and endome-

triosis (the extrauterine group irrespective of location). For endometriosis the age ranged from 19 to 32 and for adenomyosis from 30 to 45 years among a study of 40 cases. The 2 conditions might actually be treated as 2 separate diseases. It is suggested that they may be of different origins although both conditions may be activated as a result of excessive hormonal secretion of the ovaries. The fetal origin of adenomyomas is suggested. —M. E. H.

Traitement des endométriosis par l'hormone mâle. [The Treatment of Endometrioma by Male Hormone.] MOULONGUET, P. [Paris, France] *Presse méd.*, **52**:194-195. 1944.

Report of 7 cases of enometriosis successfully treated by prolonged administration of testosterone.—C. A.

Endometriosis. Two Hundred Cases Considered from the Viewpoint of the Practitioner. FALLON, J., BROSNAN, J. T., and MORAN, W. G. [Fallon Clin., Worcester, Mass.] *New England J. Med.*, **235**:669-673. 1946.

The triple action of endometriosis (as neoplasm, chemical irritant, and presumptive hormone manufacturer) makes almost any symptom possible. However, a basic syndrome has been identified, such as cumulatively increasing pain at the time of the menstrual period occurring after about 5 years of menstruation without pregnancy. Endometriotic nodules are pathognomonic but often not large enough to be felt. Because the disease can be seen long before it can be palpated, surgical exploration is desirable so that lesions can be excised before this becomes mechanically impossible. Of the 200 patients described, about half were treated by extirpation of lesions, and the other half by castration. A few young girls with extensive lesions were treated by removal of all lesions possible, plus temporary castration by a small dose of radium. Some castrated patients had recurrences as a result of stilbestrol administration.—M. H. P.

The Problem of Secondary Infection in Carcinoma of the Cervix. GARCIA, M., and SCHLOSSER, J. V. [Charity Hosp., and Tulane Univ., New Orleans, La.] *Radiology*, **46**:448-457. 1946.

A group of 449 cases of carcinoma of the cervix treated at Charity Hospital is analyzed from the standpoint of infection. Thirty-one per cent of them showed febrile reactions that were considered significant. The type of infection, its relation to treatment and stage of the lesion, and age and color of the patient as well as the 3 year end results, and the influence of completeness of therapy, are analyzed and charted. In recent cases, penicillin appears to be a potent weapon in combating infection.—R. E. S.

Indications and Limitations of Transvaginal Roentgen Therapy for Cancer of the Cervix. ERSKINE, A. W. [Cedar Rapids, Iowa] *Radiology*, **46**:458-459. 1946.

The transvaginal method of roentgen treatment of cancer of the cervix is considered to be the most efficient method of destroying the primary tumor. The author has previously reported his method which is that of retracting the vaginal walls. A dose of 6,000 r can be delivered to the tissues at a depth of 3 cm. without producing necrosis of the surface of the cervix. Either 200 kv. or 135 kv. therapy apparatus can be used and adapted to the method. Contraindications to this type of treatment are vaginal atresia, occasionally vaginismus and narrowness of the vagina. Pelvic infection may postpone such treatment and it is questionable if apparently hopeless cases should be subjected to this procedure.—R. E. S.

The Treatment of Carcinoma of the Cervix. DEL REGATO, J. A. [Ellis Fischel State Cancer Hosp., Columbia, Mo.] *Radiology*, **46**:579-5882. 1946.

In a general summary of the treatment of carcinoma of the cervix the author discusses the treatments of choice and survival rates for the various stages of the disease. In Stage I, which comprises only 10% of all cancers of the cervix, either thorough internal and external radiation or surgery now offers good survival rate. In Stage II the survival rate falls rapidly but adequate internal and external radiation is the method of choice. A combination of radiation and surgery in this stage does not seem justified for survival rate is not appreciably increased. In Stage III thorough external irradiation is the most important single factor in treatment. In Stage IV a few cases will be salvaged by careful and thorough external radiation.—R. E. S.

MALE GENITAL TRACT

Tumor of the Testicle: Analysis of One Hundred Cases: A Preliminary Report. LOWRY, E. C., BEARD, D. E., HEWIT, L. W., and BARNER, J. L. [M. C., A. U. S.] *J. Urol.*, **55**:373-384. 1946.

One hundred cases of testicular tumor in an army general hospital are reviewed. The follow up period varies from a few weeks to 3 years. Testicular tumors represented about 70% of all genitourinary neoplasms and 7.86% of all malignant tumors admitted to this hospital. A classification is presented and the pathology summarized. Painless swelling of the testicle was the most common symptom. Diagnosis was usually correctly made on the basis of physical examination. Hormone assays of the urine were not found to be of value. Treatment was by high inguinal orchiectomy followed by deep x-ray therapy. Of 100 cases 68 are well without evidence of disease. X-ray therapy was felt to have little if any curative value in cases where metastases had already occurred but was always given for possible palliation.—W. F. W.

Lymphosarcoma of the Testicle; Report of a Case. MATHÉ, C. P. [Southern Pacific Gen. Hosp., San Francisco, Calif.] *J. Urol.* **55**:530-541. 1946.

A case of lymphosarcoma of the testicle in a 63 year old male is reported. Careful examination failed to reveal any other primary site or metastasis.—W. F. W.

Hemangioma of the Testes in an Infant. ROSENTHAL, A. A. [Polyclinic Hosp., and Med. Sch., New York, N. Y.] *J. Urol.*, **55**:542-544. 1946.

A case of hemangioma of the testis in an infant of 3

months is presented. Orchiectomy was necessary.—W. F. W.

Gonocytoma. Homologous Ovarian and Testicular Tumors. I. With Discussion of "Mesonephroma ovarii" (Schiller: Am. J. Cancer 1939). TEILUM, G. [Univ. Inst. of Path. Anat., Copenhagen, Denmark] *Acta path. et microbiol. Scandinav.*, **23**:242-251. 1946.

This is a report on the congruity between ovarian tumors (Schiller's mesonephroma) and adenopapilliferous, solid or cystic tumor of the testis. The gonocytoma, here defined, is "the intermediate form of the seminoma series" and is characterized histologically by its origin from the germ cells in the testis or from homologous remnants of the medullary cords in the ovary. Case reports of testicular tumors are given, one of which exhibited many characteristics of an ovarian tumor. In contrast to the tumor described by Schiller, the growth here presented must be regarded as a true mesonephroma.—E. B. B.

Arrhenoblastoma—Androblastoma. Homologous Ovarian and Testicular Tumors. II. Including the So-Called "Luteomas" and "Adrenal Tumors" of the Ovary and the Interstitial Cell Tumors of the Testis. TEILUM, G. [Univ. Inst. of Path. Anat., Copenhagen, Denmark] *Acta path. et microbiol. Scandinav.*, **28**:252-263. 1946.

A feminizing tumor of the testis is decribed, which exhibits complete morphological congruity with the virilizing arrhenoblastoma of the ovary described by Meyer. The patient, a man aged 53, had had a testicular tumor for 30 years. For 3 years he had been impotent and during the last year gynecomastia had developed. This condition subsided after removal of the tumor. Histological examination of the growth revealed considerable lipoid content. The author concludes that testicular androblastomas should be considered as hormone-producing tumors showing all known stages of differentiation from the arrhenoblastoma. Tumors that are virilizing in women may also be feminizing in men, a fact that corresponds to previous findings concerning tumors of the adrenal cortex.—E. B. B.

A Study of the Effect of Stilbestrol Therapy on the Size of the Benignly Hypertrophied Prostate Gland. PEIRSON, E. L., [Salem, Mass.] *J. Urol.*, **55**:73-78. 1946.

By the use of a Foley catheter in the bladder and a balloon in the rectum, both bags distended with sodium iodide, the effect of stilbestrol therapy on the anterior-posterior diameter of the benignly hypertrophied prostate was studied from appropriate x-rays. In 10 of 13 cases this diameter was reduced 5 to 19 mm. In 3 cases there was no appreciable change in size. Five cases showed a decrease of 10 mm. or more. The average reduction for all 13 cases was 7 mm., representing an average reduction of 12%. There was little or no apparent reduction in the vertical diameter. These objective observations appear much more significant than any attempt at evaluation of clinical improvement or rectal findings. The author concludes that the general use of stilbestrol therapy is not warranted in benign prostatic hypertrophy.—W. F. W.

Primary Sarcoma of Prostate Gland in a Soldier. NEWMAN, H. R. [AAF Regional Station Hosp., Hunter Field, Ga.] *J. Urol.*, **55**:295-297. 1946.

Report of a case.—W. F. W.

Questionable Cancer of the Prostate Gland: Clinical Versus Routine Pathologic Evidence. LAZARUS, J. A. [New York, N. Y.] *J. Urol.*, **55**:618-625. 1946.

The author discusses the problem of the proper course of action where there are clinical signs of prostatic cancer, but where pathological examination does not confirm the clinical diagnosis. Discrepancies between clinical and pathologic data in certain cases of prostatic cancer may depend upon: (1) failure to cut sections from an adequate number of tissue blocks, and (2) failure of the pathologist to receive tissue from the part of the prostate harboring the carcinoma. The exclusion of the so-called "false capsule" of the gland in prostatectomy is the principal cause of failure to remove the carcinomatous area. This is particularly true in suprapubic and transurethral prostatectomies. The author advises castration with or without estrogen therapy in case of clinical prostatic cancer even though the pathologist may be unable to substantiate the diagnosis.—W. F. W.

Primary Carcinoma of the Prostate of Twelve Years Duration; Case Report. FLYNN, J. F. [Army and Navy General Hosp., Hot Springs, Ark.] *J. Urol.*, **55**:626-630. 1946.

A 71 year old man who had a suprapubic prostatectomy for urinary obstruction was found to have an occult adenocarcinoma in the excised gland. Nine years later he developed pain in the bone and was found to have skeletal metastases. He survived 3 more years after castration and estrogen therapy.—W. F. W.

Liver Changes and Other Effects of Diethylstilbestrol During Treatment of the Prostate Gland Cancer. WATTENBERG, C. A. [Washington Univ. Sch. of Med., and Barnes Hosp., St. Louis, Mo.] *J. Urol.*, **55**:631-640. 1946.

A case of toxic hepatitis, apparently resulting from the use of large doses of diethylstilbestrol for the control of a prostatic cancer, is described. Changes in the breast, urethra, verumontanum, testes and prostatic carcinoma, due to diethylstilbestrol, are discussed.—W. F. W.

Carcinoma of Prostate Treated with Oestrogens. FERGUSSON, J. D. [Central Middlesex County Hosp., Middlesex, England] *Lancet*, **2**:551-556. 1946.

The author considers that most carcinomas of the prostate originate in that portion of the gland lying below and behind the verumontanum. Biopsies were carried out at intervals of from 10 days to 23 months and in a few cases over periods up to 3 years. A minimum of 1.5 to 2.0 gm. of tissue was removed on each occasion. Stilbestrol or dienestrol was given in doses of from 2 to 15 mgm. daily. The specimens were stained for acid phosphates by the method of Gomori. A few normal adult prostates do not stain but in most, the enzyme is most

abundant in the posterior portion. Carcinomatous prostates generally show a consistently large amount of enzyme throughout. In 4 cases, chemical estimations showed a fall in acid phosphatase content of the prostate under treatment. There is no simple relation between the concentration of acid phosphatase in the prostate and in the serum. In every case where the primary growth showed a positive reaction, the metastatic node was also positive. Lymph glands from many other conditions were examined and acid phosphatase was rarely found. Hence it is assumed that the presence of a considerable amount of acid phosphatase in lymph gland bearing metastases suggests strongly a primary growth in the prostate.

The author has given 40 to 50 mgm. of dienestrol daily to his patients without adverse results other than vomiting. Vertigo may occur under estrogen therapy and should be considered a warning. There may be almost complete regression of the primary growth. The survival period is prolonged by estrogen treatment. A graph is given showing the length of life in 23 cases treated with estrogen and 27 cases treated in other ways. Approximately one-half the deaths of patients receiving estrogen therapy were caused by delayed, and often sudden, reactivation of the growth.—E. L. K.

The Role of Bilateral Orchiectomy in the Treatment of Carcinoma of the Prostate Gland. A Report of 82 Cases. Scott, W. W., and Benjamin, J. A. [Sch. of Med. and Dent., Univ. of Rochester, Rochester, N. Y.] *Bull. New York Acad. Med.*, **21**:307-332. 1945.

A survey of 82 patients treated for carcinoma of the prostate by bilateral orchiectomy revealed a more rapid and satisfactory amelioration of metastatic symptoms than had been afforded by previous methods. However, urinary symptoms did not show as much benefit, and within 36 months most of the patients were either dead or had lost their initial gains. Because of the discouraging end results, the authors recommend a follow-up radical perineal prostatectomy in selected cases in which the response to therapy is such that there is a reasonable chance for the removal of all or almost all of the primary lesion. They conclude that: "As yet there is no substitute for radical perineal prostatectomy for cancer of the prostate gland where the patient's condition and size and location of the lesions at the time of the first examination permits its complete removal."—M. H. P.

The Estimation of Prostatic Phosphatase in Serum and its Use in the Diagnosis of Prostatic Carcinoma. Herbert, F. K. [Med. Sch., King's College; and Royal Victoria Infirmary, Newcastle-upon-Tyne, England] *Quart. J. Med.*, **39**:221-241. 1946.

Estimations of serum acid phosphatase were made on 87 patients with prostatic carcinoma, 95 of prostatic hypertrophy, and 153 of a wide variety of diseases. Abnormally raised titers (5-281 units) were found in most of the prostatic carcinoma cases with metastases in bone and also in a few cases without demonstrable bone metastases. A few patients with non-prostatic disease showed titers above normal.

In order to assist diagnosis in cases showing only a slight rise in phosphatase, methods of inactivating prostatic phosphatase were studied. Prostatic phosphatase can be inactivated (a) by incubation of serum at 37° C. for 1 hour, or (b) by treatment of the serum with 2/5 volume of ethanol for a half hour at room temperature. Inactivation by alcohol treatment is recommended as a specific test for prostatic acid phosphatase. It gives an approximate quantitative measure of the prostatic fraction of the acid phosphatase and clearly distinguishes between raised titers due to prostatic phosphatase and raised titers of other origin. By the use of this test prostatic phosphatase can sometimes be detected even when the total titer of the serum is not raised.—F. L. W.

Primary Malignant Tumors of the Retrovesical Region with Special Reference to Malignant Tumors of the Seminal Vesicles; Report of a Case of Retrovesical Sarcoma. Lazarus, J. A., *J. Urol.*, **55**:190-205. 1946.

Only 3 cases of primary sarcoma (including 1 new case presented by the author) and 20 cases of carcinoma were found in a review of the literature of primary malignant tumors of the retrovesical region involving the seminal vesicles. Of the 20 carcinoma cases, the author feels that only 7 can be reasonably listed as authentic since the prostate was involved in the malignant process in 13, introducing the possibility of a primary prostatic cancer in the latter group. Brief abstracts of the recorded cases are presented. This disease is most prevalent among old men, and the left vesicle is involved slightly more frequently than the right (57.1% vs. 42.8%). Urinary symptoms ranging from increased frequency to acute urinary retention were present in about 50% of the cases where the symptomatology was noted in the protocols. In 12 of the 20 cases, statements regarding metastases were noted and of this number 66.6% occurred in the regional lymph nodes, 41% in the liver and 33.3% in the lung. The mortality in this group of cases was about 85%. —W. F. W.

Cancerous and Precancerous Lesions of the Penis: A Clinical and Pathological Study Based on Twenty-three Cases. Melicow, M. M., and Ganem, E. J. [Coll. of Physicians and Surgeons, Columbia Univ., New York, N. Y.] *J. Urol.*, **55**:486-514. 1946.

Nineteen cases of primary penile cancer constitute the basis for this report. The clinical and pathological features of cancer of the penis are discussed with respect to incidence, etiology, pathogenesis, duration of the disease, pathology, symptomatology, metastases, diagnosis, treatment, and association with other disease. Four instances of secondary penile cancer are also presented and the literature on that subject reviewed. Prophylactic circumcision to reduce the incidence of penile cancer is recommended as a routine for all healthy male babies. Routine inguinal lymph node dissection in all cases of penile cancer is advised except in patients with widespread metastases and in patients who are unable to tolerate extensive surgery for other reasons. Metastases may develop by penetration of the dorsal vein of the penis as well as by lymphatic channels. Precancerous lesions of the penis are discussed and their pathological histology demonstrated. —W. F. W.